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RED DRUM AQUACULTURE

Proceedings of a Symposium on the Culture of Red Drum and Other Warm Water Fishes

Sciaenops ocellatus

Editors

C.R. Arnold, G.J. Holt and P. Thomas

Consulting Editor
D.E. Wohlschlag

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Symposium held in
Corpus Christi, Texas
U.S.A.
June 22–24, 1987

Red Drum Research Symposium
June 22–24, 1987, Corpus Christi

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CONTENTS

Journal Title Page i
Journal Editor's Note ii
Title Page of Conference iii
Organizers & Session Chairs iv
Contents v
Preface 1
List of reviewers 2

SECTION I.

Culture and Nutrition *C.R. Arnold*, Editor

Winter survival of fingerling red drum (*Sciaenops ocellatus*) in South Carolina culture ponds. ***J. Stephen Hopkins, Theodore I.J. Smith, Alvin D. Stokes and Paul A. Sandifer*** 5

Nutritional requirements of red drum: a review. ***Edwin H. Robinson*** 11

Morphometric comparison of fingerling spotted seatrout, orangemouth corvina and their hybrids. ***Lynn S. Procarione, Timothy L. King and Britt W. Bumguardner*** 21

Growth of juvenile red drum: adenylate concentrations, RNA-DNA ratio and effects of ovine growth hormone. ***Peter Thomas, Mark E. Westerman, P.F. Dehn, E. Nowicki, G. Joan Holt and C.R. Arnold*** 29

Evaluation of etomidate as an anesthetic for red drum, *Sciaenops ocellatus*. ***W.W. Falls, G. K. Vermeer and C.W. Dennis*** 37

SECTION II.

Reproduction and Genetics *C.R. Arnold*, Editor

Induced spawning of spotted seatrout, red drum and orangemouth corvina, (family: sciaenidae) with luteinizing hormone-releasing hormone analog injection. ***Peter Thomas and N. Boyd*** 43

Population structure and genetic variation in red drum. ***John M. Wakeman and Paul R. Ramsey*** 49

Studies on the basic structure of the red drum (*Sciaenops ocellatus*) genome. ***J.R. Gold, K.M. Kedzie, D.A. Bohlymeyer, J.D. Jenkin, W.J. Karel, N. Iida and S.M. Carr*** 57

Controlled year-round spawning of red drum, *Sciaenops ocellatus*, in captivity. ***C.R. Arnold*** 65

Differing reproductive life histories between temperate and subtropical groups of *Cynoscion nebulosus*. ***Nancy Brown-Peterson and Peter Thomas*** 71

Measurements of ovarian development in red drum (*Sciaenops ocellatus*) from offshore stocks. ***Gary R. Fitzhugh, Theron G. Snider III and Bruce A. Thompson*** 79

SECTION III.

Natural History and Larval Biology. ***G. Joan Holt,*** Editor

The use of stable isotope tracers in red drum (*Sciaenops ocellatus*) culture. ***R.K. Anderson, M.L. Dickson, G. Joan Holt, D. Ritz and P.L. Parker*** 87

Growth rates and validation of age estimates of red drum, *Sciaenops ocellatus*, in a Louisiana salt marsh impoundment. ***Daniel W. Beckman, Gary R. Fitzhugh and Charles A. Wilson*** 93

A procedure for identifying sciaenid eggs. ***S.A. Holt, G. Joan Holt and L. Young-Able*** 99

Fine-scale spatial distribution of red drum, *Sciaenops ocellatus*, larvae. ***Richard F. Shaw, David L. Drullinger, Kenneth A. Edds and Deborah L. Leffler*** 109

The RNA-DNA ratio: measurement of nucleic acids in larval *Sciaenops ocellatus*. ***Mark E. Westerman and G. Joan Holt*** 117

First larval rearing trials of red drum (*Sciaenops ocellatus*) in Martinique (French West Indies) ***P. Soletchnik, E. Thouard, E. Goyard, C. Yvon and P. Baker*** 125

SECTION IV.

Stress Physiology, Disease and Environmental Requirements ***Peter Thomas,*** Editor

Handling and transport-induced stress in red drum fingerlings (*Sciaenops ocellatus*). ***J.R. Tomasso and Gary J. Carmichael*** 133

Thyroid function in red drum. ***Duncan S. MacKenzie*** 139

Temperature sensitivity of lactate dehydrogenase ($LDH\text{-}A_4$ isozyme) from white skeletal muscle of sciaenid fishes. ***Zulema L. Coppes and George N. Somero*** 147

Assessing immunocompetence of red drum (*Sciaenops ocellatus*). *D.H. Lewis and Peter Thomas* 153

Changes in prolactin cell size and chloride cell number in young red drum (*Sciaenops ocellatus*) during salinity adaptation. *Hong Y. Yan and Peter Thomas* 157

A diet induced disease in common snook, *Centropomus umdecimalis*. *M.E. Clarke, W. Sheldon, K. Dowd and M. Schmale* 165

Biophysical model of osmoregulation and its metabolic cost in red drum. *J.D. Bryan, K.D. Ham and W.H. Neill* 169

Preliminary studies on the use of chloroquine as a systemic chemotherapeutic agent for amyloodinosis in red drum (*Sciaenops ocellatus*). *D.H. Lewis, Wang Wenzing, A. Ayres and C.R. Arnold* 183

SECTION V.

Abstracts of papers not included in proceedings.

Development of marine finfish culture in Texas: intensive culture system. *C.R. Arnold* 193

Laboratory studies of red drum and larvae: a review *G. Joan Holt* 193

Development of marine finfish culutre: artifical diets for larval fish. *G. Joan Holt* 193

Dynamics of elemental and biochemical components in the early life stages of red drum (*Sciaenops ocellatus*). *W.Y. Lee, S.A. Macko, X.H. Mao and C.R. Arnold* 194

Regime spawing of red drum, *Sciaenops ocellatus*: cyclical effects on serum steriods, gonadal cytology and ultrastructure. *Daniel E. Roberts, Jr., R.O. Reese, H.J. Grier and R. MacGregor III* 194

Seasonal cycles of oocyte growth and plasma steriod hormone levels in captive orangemouth corvina broodstock. *Peter Thomas and J.A. Prentice* 195

A comparison of black drum, red drum, and their hybrid in saltwater pond culture. *Anne Henderson-Arzapalo, Robert L. Colura and Anthony F. Maciorowski* 195

Distribution of eggs and larvae of red drum (*Sciaenops ocellatus*) in Texas coastal waters. *S.A. Holt, G. Joan Holt and C.R. Arnold* 196

Development of marine finfish culture in Texas: immunization against disease. ***D.H. Lewis*** 196

Biochemical stress responses of red drum to common culture procedures. ***Peter Thomas*** 197

Evaluation of metomidate as an anesthetic for common snook, *Centropomus undecimalis*. ***G.K. Vermeer and B. Falls*** 197

PREFACE

Growing interest in the culture of red drum and other warm water marine fishes in southern regions of the United States has stimulated a marked expansion of research on the biological aspects of their culture over the past five years. A Red Drum Aquaculture Conference, comprising a joint Production Short course and a Research Symposium, was held in Corpus Christi, Texas, June 22-24, 1987 to review recent technical and scientific advances in warm water marine fish culture. More than 350 participants listened to sixty technical and scientific presentations at the meeting.

The primary objective of the Research Symposium was to draw together people actively involved in research on biological aspects of the culture of red drum and other warm water marine species. The papers were grouped in four major categories: (1) Culture and Nutrition, (2) Reproduction and Genetics, (3) Natural History and Larval Biology, and (4) Stress Physiology, Disease and Environmental Requirements. In addition to research on red drum, the Symposium included relevant work on common snook (*Centropomus undecimalis*), spotted seatrout (*Cynoscion nebulosus*), and other sciaenid fishes.

The proceedings of the Symposium consisted of short communications of original research. The method of presentation was chosen to enable important research findings to be brought to the attention of interested researchers within a reasonable time span. Research communications were accepted for publication only after passing the normal review process for *Contributions in Marine Science*. The reviewers are gratefully acknowledged on page 2.

The presentations were lively and led to many informal discussions with wide ranging exchanges of ideas, indicating that the Symposium was a success. It is hoped that further research on the aquaculture of red drum and other warm water marine species will be stimulated by these discussions and the research reported in these proceedings. Appreciation is due to Drs. D.H. Lewis and W.H. Neill, Texas A&M University, and Mr. R.L. Colura, Texas Parks and Wildlife Department, who were co-organizers of this meeting. Gratitude is also expressed to all those who chaired the sessions (see p. 000) and to the many staff of the University of Texas Marine Science Institute who assisted in the preparations. Funding for this publication was provided by the University of Texas at Austin. We wish to thank Dr. Gerhard J. Fonken, Executive Vice President and Provost, The University of Texas at Austin for his support.

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SECTION I

Culture and Nutrition

C.R. Arnold, Editor

Contributions in Marine Science. (1988) Supp. to Vol. 30:5–10.

WINTER SURVIVAL OF FINGERLING RED DRUM (*SCIAENOPS OCELLATUS*) IN SOUTH CAROLINA CULTURE PONDS

J. Stephen Hopkins, Theodore I.J. Smith, Alvin D. Stokes and Paul A. Sandifer

Waddell Mariculture Center
P.O. Box 809
Bluffton, South Carolina 29910

ABSTRACT

Fingerling red drum, spawned in September, were restocked into culture ponds at densities of 9000, 18000 and 36000 fish/ha in December, 1985. The ponds were harvested in April, 1986. A significant difference in survival was noted between the various stocking densities with the stocking density being inversely proportional to the survival rate. Mean survivals for the three density treatments were 80.7, 55.8 and 42.3%. During the winter, the fish did not appear to be actively consuming the compounded ration provided and zooplankton levels were low. The influence of density upon survival may indicate that the poor survival is related to food availability rather than temperature per se.

INTRODUCTION

Over the past several years there has been a growing interest in the cultivation of red drum. In South Carolina, research is underway to develop commercial cultivation techniques and evaluate the potential impact of a stock enhancement program.

Since the natural spawning season for red drum is in the fall, this would seem to be the logical time to spawn captive broodstock. However, it is becoming apparent that there are logistical problems associated with the production of juvenile fish from autumn spawning as opposed to those spawned in the spring (Hopkins 1987).

Larger red drum tolerate temperatures as low as 2°C in the wild (Simmons and Breuer 1962). Winter survival of large juveniles which have been weaned to compounded rations is excellent in salt water even though the temperature may drop to 4°C (Hopkins, Stokes, Sandifer, Smiley and McTeer 1986). The lower lethal temperature for fingerlings was reported to be 6.9°C at 5‰ salinity and higher for salinities below 5 and greater than 10‰ (Procarione and Neill 1987). Larvae less than two weeks old were inactive and were not seen catching prey when temperatures were only as low as 20°C (Holt, Godbout and Arnold 1981).

METHODS

This work was conducted by the South Carolina Marine Resources Division at the Waddell Mariculture Center field station near Bluffton, South Carolina. The center draws its water from the adjacent high salinity estuarine embayment off Port Royal Sound near its entrance to the ocean.

Captive broodstock were tank-spawned without the use of hormones or handling in late September using ambient temperatures. Three-day-old sac fry were stocked into a 0.5 ha pond at a rate of one-million per hectare. The pond had been fertilized and filled two weeks prior to stocking with organic and inorganic fertilizers to induce a phytoplankton bloom. Salinities were 28 ± 2‰ over the course of the fingerling production period. Fry were stocked at a rate of one-million per hectare. Nine weeks later, 0.48 gram fingerlings were harvested.

A portion of the fingerlings were hand-counted and restocked into 0.1 and 0.5 ha ponds at rates of 9000, 18000 and 36000 ha. The ponds do not have identical previous usage although all were thoroughly dried and the bottoms plowed to oxidize the organic material prior to refilling. Two 0.1 ha and one 0.5 ha pond were stocked with 9000 fish ha. Two 0.1 ha ponds were stocked with 36000 fish ha.

Feeding with salmon starter crumbles (Zeigler Brothers, Inc., Gardners, Pennsylvania) was begun two weeks after stocking. The ponds stocked at 9000, 18000 and 36000 were fed 1.5, 3.0 and 6.0 kg ha day respectively.

Over the course of the study the salinity ranged from 20 to 34‰. Temperatures were recorded at dawn from a dissolved oxygen meter and ranged from 4 to 25°C. The temperature and salinity profiles are depicted in Figure 1.

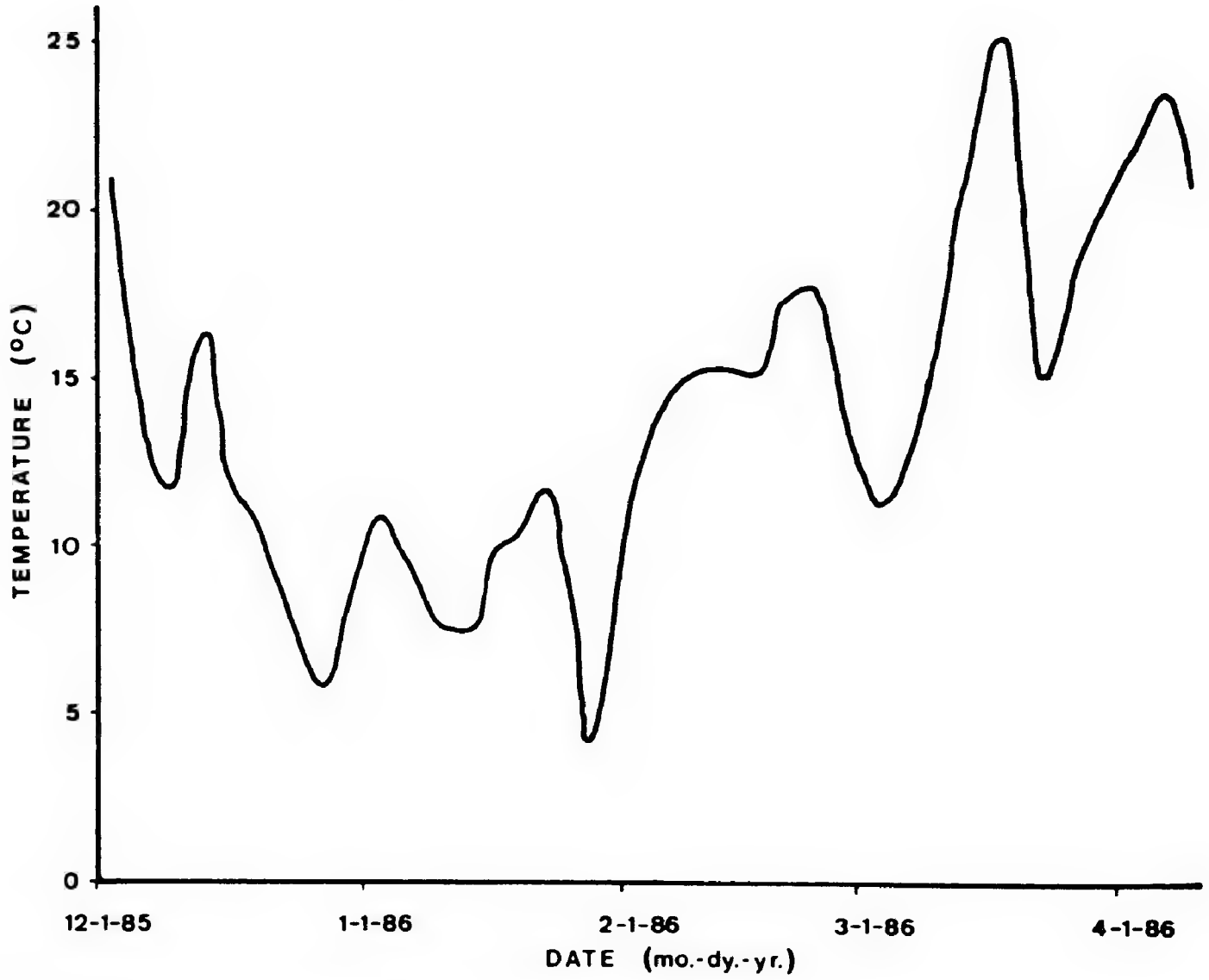

FIG. 1. Temperature and salinity profiles for ponds.

At monthly intervals, a sample was removed from each pond with a seine and the fish individually weighed. Zooplankton was not monitored after stocking.

There was little, if any, bird predation during the course of the study. At harvest, no extraneous organisms which could be considered predators on red drum fingerlings were found.

RESULTS

The growth rate of fingerlings after transferal to the density study ponds was slow. Mean weights of the various replicates of each treatment are provided in Table 1. During January, the mean weight actually decreased in many ponds. No significant differences were found in the mean size of fish from the various treatments.

During the coldest portions of the winter, dead fish were noted in the density experiment ponds as well as in the unharvested fingerling ponds. The dead fish as well as those in the monthly seine samples had concave abdomens and appeared to be starving. While not enumerated on a regular basis, zooplankton was scarce during this time. Phytoplankton levels were also very low and the secchi disc visibilities were generally well in excess of 1 meter. The water clarity facilitated direct observation of the fish. Few fish were seen consuming the salmon starter ration being provided, and this, in conjunction with the poor condition of the fish indicated that most were not eating.

Survival of fingerlings from early December, 1985 until early April, 1986 averaged 57.48% overall with a range of 22.7 to 85.3% (Table 1). A Student's t test (Steel and Torrie 1980) indicated that survival differences between the 9000 and 36000/ha stocking rates were significant ($t = 5.70$ for 2 d.f.).

The presence of dying fingerlings in the ponds through the winter, when zooplankton levels are low, indicates that fingerlings which are not weaned onto dry rations may suffer mortalities through starvation or a combination of starvation and an intolerance of low temperatures. The relationship between fingerling density and overwinter survival indicates that the availability of naturally occurring prey items may be a major factor (Table 2). While naturally occurring food items (benthos, zooplankton) were not enumerated, it is possible that these items were cropped to a greater extent, and thus became less available, in the high-density ponds.

CONCLUSIONS

The effect of density upon winter red drum survival in ponds may indicate that the relatively low survival rate was not a function of temperature intolerance but rather a function of the availability of natural prey items for fish which have not been weaned onto formulated rations. This aspect of red drum production should be further investigated in a situation where the concentration of prey items can be manipulated and closely monitored.

In previous and subsequent studies on red drum grow-out in these ponds, the winter survival of larger juveniles which had become accustomed to

TABLE 1

Monthly growth of red drum in culture ponds at various densities

DATE (y/m/d)	TREATMENT (fish/ha)	REP (#)	MEAN WT (gram)	STD. DEV.
851203	9,000	1	0.48	0.26
860103	"	"	0.55	0.20
860203	"	"	0.80	0.40
860304	"	"	1.20	0.40
860403	"	"	1.92	0.41
851203	9,000	2	0.48	0.26
860103	"	"	0.61	0.14
860203	"	"	0.56	0.14
860304	"	"	1.76	0.54
860403	"	"	2.98	0.50
851203	9,000	3	0.48	0.26
860103	"	"	0.47	0.21
860203	"	"	0.50	0.13
860304	"	"	1.52	0.57
860403	"	"	4.30	1.36
851203	18,000	1	0.48	0.26
860103	"	"	0.59	0.31
860203	"	"	0.51	0.14
860304	"	"	1.27	0.85
860403	"	"	1.57	0.54
851203	18,000	2	0.48	0.26
860103	"	"	0.53	0.14
860203	"	"	0.46	0.11
860304	"	"	1.49	0.68
860403	"	"	2.10	0.80
851203	36,000	1	0.48	0.26
860103	"	"	0.61	0.18
860203	"	"	0.58	0.15
860304	"	"	1.16	0.52
860403	"	"	1.93	0.69
851203	36,000	2	0.48	0.26
860103	"	"	0.80	0.34
860203	"	"	0.65	0.40
860304	"	"	1.92	0.68
860403	"	"	3.40	1.23
851203	36,000	3	0.48	0.26
860103	"	"	0.70	0.30
860203	"	"	0.75	0.23
860304	"	"	2.08	0.56
860403	"	"	7.39	2.29

TABLE 2

Summary of growth and survival of red drum fingerlings overwintered in culture ponds at various densities

REP. NO. (#)	POND SIZE (ha)	STOCKINGS DENSITY (#/ha)	STOCKING SIZE (gram)	HARVEST SIZE (gram)	HARVEST SURVIVAL (%)
1	0.1	9,000	0.5	1.9	83.0
2	0.1	9,000	0.5	3.0	85.3
3	0.5	9,000	0.5	4.3	73.7
	mean	9,000	0.5	3.1	80.7
1	0.1	18,000	0.5	1.6	46.1
2	0.1	18,000	0.5	2.1	65.4
	mean	18,000	0.5	1.8	55.8
1	0.1	36,000	0.5	1.9	43.5
2	0.1	36,000	0.5	3.4	22.7
3	0.5	36,000	0.5	7.4	60.7
	mean	36,000	0.5	4.2	42.3

accepting pelleted rations was excellent, generally in excess of 85% (Hopkins *et al.* 1986, Sandifer, Stokes, Hopkins, Smiley and Smith, in preparation). In these other trials, the juveniles were larger and before the onset of winter temperatures. Thus, survival of larger juveniles which are accustomed to accepting a pelleted ration does not appear to be a problem.

This work has had ramifications in the development of a production scenario of commercial red drum farming. With poor survival of unweaned fingerlings through the winter, the farmer is advised to spawn fish in the spring and stock grow-out ponds in early summer. In addition, this work may further substantiate the hypothesis that the early onset of low temperatures may adversely effect the subsequent year-class (Holt *et al.* 1981).

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NUTRITIONAL REQUIREMENTS OF RED DRUM: A REVIEW

Edwin H. Robinson

Mississippi State University
Delta Branch Experiment Station
Stoneville, Mississippi, 38776

ABSTRACT

Numerous studies have documented the feeding habits of red drum, *Sciaenops ocellatus*, in nature. Based on inferences drawn from these studies and on data from nutritional studies with other fish, red drum are expected to require the same nutrients for normal metabolic function as other fish. Quantitative requirements, which can be used for feed formulation, are available for protein, energy, lipid, phosphorus, and lysine. Red drum feeding habits and nutritional requirement studies are reviewed and feed formulation is discussed.

INTRODUCTION

Based on nutritional data derived from studies with terrestrial animals and fish, there appears to be a qualitative uniformity of nutritional requirements among vertebrates (Cowey and Tacon 1981). That is, a source of nonspecific nitrogen, essential amino acids, essential fatty acids, vitamins, minerals, and water as well as a source of energy are required for the normal metabolic function of animals.

Quantitative nutritional requirements for macro- and micronutrients have been reported for a number of fish (NRC 1981, 1983). These requirements are affected by such factors as species, size, age, sex, environment, dietary nutrient interactions, nutrient availability from feedstuffs, presence of toxins or inhibitors in feeds, desired level of performance, and desired carcass composition. It is thus difficult to determine an exact quantitative requirement that is optimum for all circumstances. Often, a requirement must be inferred in order to formulate feeds for various life stages or for species for which no nutritional information exists. Since nutritional requirements form a basis for formulating feeds that are adequate in nutrients and are without detrimental imbalance, it is advantageous to determine the nutritional requirements for each species of interest as well as for the various life stages of a species.

The information presented herein summarizes studies on feeding habits and nutritional requirements of red drum. (Feed formulation is discussed.)

FEEDING HABITS

Fish may prefer specific feed items, but generally consumption is a function of food availability (Lagler, Bardach and Miller 1962). Even so, studies of

feeding habits provide information on dietary preferences as well as give insight into the nutrient needs of a species. Fish can be classified broadly on the basis of their feeding habits into detritivores, herbivores, carnivores, and omnivores (Moyle and Cech 1982). In general, carnivorous fish require higher levels and better quality protein than herbivores or omnivores. Omnivores probably have an intermediate protein requirement, whereas a detritivore, which feeds low on the food chain, might be expected to have a lower protein requirement than that of the other groups, and less need for high-quality protein. Although these inferences do not always hold, they are useful guidelines that can serve as a basis for feeding species for which no nutritional data are available.

Initial information concerning the feeding habits of the red drum was generally gathered incidentally as a part of other studies (Linton 1904, Hildebrand and Schroeder 1928. Pearson 1929, Gunter 1945, Kemp 1949, Miles 1949, Knapp 1950, Reid 1955, Reid, Inglis and Hoese 1956, Darnell 1958). Although these studies were based on observations of a relatively small number of fish, they indicated the carnivorous nature of red drum and demonstrated that crustaceans and fish were the major food items consumed by red drum. Yokel (1966) conducted studies of red drum feeding habits in South Florida and found shrimp to be the most important food item for red drum during July to September, while crabs were the most important food item during other periods. He also found that larger red drum consumed proportionally more crabs than did smaller red drum. Boothby and Avault (1971) studied the food habits of red drum in southeastern Louisiana. They examined 286 adult red drum that had identifiable food substances in the stomach and found that fish was the main food item during the winter and spring. Fourteen different species of fish were identified, but menhaden was the predominant species consumed. Crustaceans comprised the bulk of the diet during the summer and fall months. The predominate single species of Crustacea was the blue crab. Penaeid shrimp occurred more frequently than other species of shrimp. Overstreet and Heard (1978) examined 107 large red drum collected from the Mississippi Sound during the period of May, 1976, through August, 1977. They found the predominant food items to be blue crabs and shrimp. Proportionately greater numbers of shrimp were consumed in the fall and winter whereas blue crabs were the primary dietary item in spring and summer. Fish were the next most abundant food item followed by polychaete worms.

Few food habit studies of red drum have concentrated on juveniles. Bass and Avault (1975) examined 568 juvenile red drum, 8.0 to 183 mm standard length (SL), collected from Louisiana waters. They reported that generally the most abundant organisms of an edible size were utilized most heavily. Also, their data indicated that fish of less than 15 mm SL fed primarily on zooplankton, fish from 15 to 75 mm SL fed on bottom invertebrates and young of other fish, and fish greater than 75 mm SL fed primarily on decapods and fish. More recently, Steen and Laroche (1983) studied 222 specimens of red drum larvae and early juveniles (1.8 to 12.6 mm SL) taken from the Mississippi Sound and the northern Gulf of Mexico. They found that copepods and

crustacean nauplii dominated the diets of most fish. Crustacean eggs and decapod post-larvae were other important food items. The smallest larvae, 1.8 to 3.0 mm SL, fed primarily on copepod nauplii and eggs. Larger larvae, 3.0 to 8.5 mm SL, fed primarily on copepodites, however, copepod eggs were an important food item. The largest larvae (8.5 to 13 mm SL) fed primarily on decapod post-larvae, copepodites, and polychaetes. As red drum larvae grew, they fed on increasingly larger prey.

Though there are some differences reported from the various food habit studies, the results are generally similar. Most of the data indicate that although red drum are highly carnivorous, they are somewhat indiscriminate in their food preferences as evidenced by seasonal changes in feeding habits. There is some evidence that size, at least in small fish, may influence food selection, which is most likely simply a matter of being able to consume an available prey species.

Since the natural diet of red drum contains a liberal amount of high-quality protein, it can be assumed that feeds formulated for red drum should be high in protein and that some animal protein should be included in order to meet amino acid requirements and perhaps improve palatability. Further, because their natural diet is low in carbohydrates and high in protein it might be concluded that red drum would not digest and assimilate carbohydrate efficiently and that protein would be efficiently utilized as an energy source. If these inferences are correct, feed cost should be a considerable portion of production costs in red drum culture.

NUTRIENT REQUIREMENTS

Protein and Amino Acids

Proteins are the major organic component in animal tissue, making up approximately 65-80% of the total dry tissue weight (NRC 1983). Red drum, like other animals, do not actually require protein but have a requirement for amino acids and nonspecific nitrogen. The most economical source of these compounds is a mixture of protein. A continuous supply of protein is needed for synthesis of new protein (as during growth and reproduction) and to replace existing proteins (maintenance). A deficiency of protein results in a reduction or cessation of growth or a loss of weight.

Protein Requirements

There has been little research conducted to determine the protein requirement of red drum. Lin and Arnold (1983) fed a series of semi-moist diets, that ranged in protein from 30% to 50%, to juvenile red drum reared in saltwater. Fish fed diets containing 30% and 35% protein did not grow or convert feed as well as fish fed higher levels of dietary protein. Also, survival of fish fed the 30% and 35% protein diets was only 50% compared to 100% survival of fish fed diets containing higher protein levels. In a second trial, these researchers fed diets containing either 40%

or 50% protein to juvenile red drum and concluded that 50% dietary protein was optimum.

Daniels and Robinson (1986) investigated the protein and energy requirements of 4 to 5 g juvenile red drum reared in water of approximately 6‰ salinity. Menhaden fish meal and casein were the protein sources used in the experimental diets. Two experiments were conducted. The results of the first experiment, which was conducted at a temperature range of 22 to 26°C, indicated that 35% dietary protein was adequate for good weight gain and acceptable body composition, that is, a low fat and high protein carcass. The results of the second experiment, which was conducted at a temperature range of 25 to 33°C, indicated that 45% dietary protein was required for maximal growth. The authors concluded that the lower protein requirement determined in their study compared to that reported by Lin and Arnold (1983) may have been due to differences in salinity, diet composition, or water temperature.

The protein requirement reported for red drum is in the range (31% to 56% dietary protein) reported to be required by other fish (NRC 1983). It is difficult to recommend an appropriate protein level for each set of culture practices and environmental conditions because numerous factors affect the protein requirement. However, a dietary protein level of 45 to 50% for juvenile red drum and a dietary protein level of 35 to 45% for advanced fingerlings and adult red drum should be adequate.

Amino Acid Requirements

Nutritionally, the various amino acids can be divided into two groups, indispensable (essential) and dispensable (nonessential). An indispensable amino acid is one that the animal cannot synthesize, or cannot synthesize it in sufficient quantities to support maximum growth. Indispensable amino acids must be supplied preformed in the diet. Most animals, including fish, require the same 10 indispensable amino acids. These are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (NRC 1983). The dispensable amino acids are those that can be synthesized by the animal. Although there has been no work to determine the qualitative amino acid requirements of red drum, it is assumed that red drum require the same 10 amino acids as other fish.

Robinson, Brown and Davis (1987) investigated the lysine requirement of red drum. These researchers fed practical-type diets supplemented with crystalline L-amino acids to juvenile red drum reared in water of 6‰ salinity. Based on weight gain, feed conversion, and serum free lysine concentrations, they reported the lysine requirement for red drum to be between 5.3 and 6.6% of the dietary protein. The lysine requirement for various other fish ranges from 4.4 to 5.8% of the dietary protein (NRC 1983), thus the lysine requirement for red drum is similar to those recommended for other fish. There have been no other amino acid studies reported for red drum; however,

diets formulated for red drum which contain liberal amounts of animal protein will likely meet or exceed amino acid requirements. Recommended levels of animal protein are discussed under feed formulation.

Energy

Relatively little information is available on energy requirements of fish. Energy research has not been given priority because an excess or deficiency of energy will not affect the health of fish appreciably, and ingredients used in practical diets are not likely to be extremely high or low in energy. Even so, providing the necessary energy in a fish feed is important because a deficiency of energy means that protein and other nutrients will not be utilized efficiently and too much energy in the ration can limit the daily intake of food and can cause excessive fat deposition (Lovell 1984a).

There has been one study reported concerning the energy requirements of red drum. Daniels and Robinson (1986) studied the protein and energy requirements of juvenile red drum reared in 6‰ salinity water. They conducted two experiments in which the diets varied in protein and energy. The energy levels used in the study were 3.4, 3.8, 4.3, and 4.7 kcal g of diet. An estimate of digestible energy was made by subtracting the energy contributed by dietary fiber from the gross energy of the diet. Their data indicated that approximately 3.8 kcal/g of diet was adequate for maximum weight gain and a desirable body composition. Expressed on kcal/g of dietary protein, optimum energy ranged from 8.5 to 10.9. This is similar to the dietary energy recommended for catfish (Lovell 1984b).

Carbohydrate

Fish apparently do not have a carbohydrate requirement, but warm water fish are generally able to use high levels of carbohydrate as an energy source (NCR 1983). This is advantageous since carbohydrate is an inexpensive energy source and can be used to spare the more expensive protein component, that is, carbohydrate can be used for energy allowing protein to be used for tissue growth.

Practical feeds for warm water fish contain up to 35 or 40% carbohydrate (Robinette 1984). There have been no studies that concentrated on the levels of dietary carbohydrate that can be utilized by red drum. However, experimental diets ranging in digestible carbohydrate from 1 or 2% to greater than 35% have been fed to red drum in experiments designed to study other nutrient requirements (Daniels and Robinson 1986, Davis and Robinson 1987, Williams and Robinson 1987). Based on these studies, it appears that red drum can utilize relatively high levels of dietary carbohydrate without adverse effects. This means that considerable amounts of grain products can be used in commercial red drum feeds, which should help to reduce feed cost.

Lipid

A balanced diet for fish should include lipids, both for energy and to provide essential fatty acids (Stickney 1984). The linolenic and/or linoleic families of fatty acids have been shown to be essential for various fish (NRC 1983). Fish have been fed dietary lipid ranging from less than 1.0% to greater than 15% with minor differences in growth; however, high levels (15% or greater) generally resulted in reduced weight gain (NRC 1983). Also high levels of dietary lipid may result in undesirable deposition of fat in fish tissues. Generally, warm water fish feeds used for grow-out contain less than 8% lipid.

Only one lipid study has been reported for red drum. Williams and Robinson (1987) reared juvenile red drum in 6‰ salinity and fed them diets containing graded levels of lipid ranging from 1.7 to 18.7%. All diets contained approximately 1.0% lipid from shrimp-head meal, with the remainder of the lipid being supplied by menhaden oil. Weight gain, feed conversion, and survival were best at dietary lipid levels of 7.4 and 11.2%. Fish fed diets containing 15% or greater lipid had lower weight gains and higher feed conversion ratios than fish fed diets containing lower levels of lipid. Whole-body lipid concentrations increased as dietary lipid increased up to 7.4%. Whole-body fatty acids generally reflected dietary fatty acids. It appears that a level of dietary lipid near 7% is adequate for red drum.

There is no indication of which lipid source is best for use in red drum diets. However, Daniels and Robinson (1986) used both menhaden and soybean oil in red drum diets up to a level of 12% of the diet, without adverse affects. A mixture of oils from fish and plant sources would probably be adequate for red drum.

Vitamin Requirements

The need for vitamins in fish feeds was recognized more than 40 years ago. However, it has only been in the past decade or so that many of the vitamin requirements for fish have been determined. Presently 14 vitamins have been shown to be necessary for the normal metabolic function of fish (NRC 1983). There have been no vitamin requirement studies conducted with red drum. It is assumed that the vitamin requirements listed by the National Research Council (NRC 1983) for warm water fish will be adequate for red drum. The vitamins and recommended amounts for use in channel catfish feeds are given in Table 1 (Robinson 1984). These values may be useful as guidelines for formulating red drum diets.

Mineral Requirements

Red drum probably require the same minerals as warm blooded animals for tissue formation and various metabolic processes. Additionally, red drum, as other fish, use inorganic elements to maintain osmotic balance and can

TABLE 1

Recommended amounts of vitamins to be added in extrusion processed feeds for channel catfish

Vitamin	Amount per kg(in mg)
Thiamin	11
Riboflavin	13.2
Pyridoxine	11
Pantothenic acid	35.2
Nicotinic acid	88
Vitamin B-12	0.09
Choline chloride (70%)	550
Ascorbic acid	375.6
Vitamin A	4,400 (IU)
Vitamin D_3	2,200 (IU)
Vitamin E	55
Vitamin K	11

probably use environmental ions to meet requirements for certain minerals, *e.g.* calcium.

The dietary phosphorus requirement for red drum has been determined (Davis and Robinson 1987). Juvenile red drum were fed diets containing graded levels of inorganic phosphorus supplied as sodium phosphate. Results indicate that 0.86% dietary phosphorus was needed for optimum tissue mineralization. There have been no other mineral requirement studies conducted with red drum. It in assumed that the mineral requirements summarized by the National Research council (NRC 1983) for warm water fish are adequate for red drum. Mineral premixes (modified from Lovell, 1984b) for channel catfish should provide the following amounts of minerals in the feed (mg/kg): Mn, 25; I, 5; Cu, 5; Zn, 150; Fe, 30; Co, 0.05; and Se, 0.1. These values may be useful as guidelines for formulating red drum diets.

FEED FORMULATION

Information on nutritional requirements is essential to the development of efficient least-cost feeds for red drum culture. At this time, feeds can be formulated specifically for red drum based upon feeding habits, the nutritional requirements that have been determined, and on nutritional data derived from studies with other fish. Restrictions to feed formulation (in addition to nutrition) may be a function of processing or milling, inherent problems with certain feedstuffs, or other miscellaneous factors. It appears that feed ingredients commonly used in commercial warm water fish feeds are satisfactory for use in red drum feeds. Studies conducted at Texas A&M

University indicate that red drum require some animal protein (12 to 15%) in the feed; that is, they do not consume diets formulated with all plant protein (Robinson, Daniels, Williams and Wurts 1984). Also, the inclusion of shrimp meal in the diet appears to improve palatability. Further, it is assumed that vitamin and mineral premixes listed for warm water fish by the National Research Council (NRC 1983) or those used in catfish feeds are adequate for red drum. An example of a feed formulation that could be used for grow-out of red drum is given in Table 2. This formulation is based upon a limited amount of nutritional data derived specifically from studies with red drum, thus refinements will be necessary as new information becomes available.

TABLE 2

Example of a 37% crude protein feed for grow-out of red drum from approximately 6 inches to harvest.[1]

Ingredient	Percentage (as fed)
Soybean meal (48%)	50
Menhaden fish meal	12
Shrimp-head meal	5
Ground corn	28.9
Vitamin premix[2]	0.1
Mineral premix[2]	0.1
Fat[3]	1.5
Dicalcium phosphate	2.4

[1] Based on available data on red drum nutrition. Feed formulation is for a floating feed. A sinking feed can be made with slight modifications in the formulation or in processing methods. Wheat or milo can probably be used in place of corn.
[2] Based on catfish premixes. Amount added will depend on premix manufacturer.
[3] Spray on finished pellet.

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MORPHOMETRIC COMPARISON OF FINGERLING SPOTTED SEATROUT, ORANGEMOUTH CORVINA, AND THEIR HYBRIDS

Lynne S. Procarione, Timothy L. King and Britt W. Bumguardner

Texas Parks and Wildlife Department
Perry R. Bass Marine Fisheries Research Station
Star Route Box 385
Palacios, Texas 77465

ABSTRACT

Artificial propagation of spotted seatrout *Cynoscion nebulosus* and orangemouth corvina *Cynoscion xanthulus* has resulted in spawning and saltwater pond culture of the parent species, a spotted seatrout female × orangemouth corvina male hybrid, and the reciprocal hybrid. Subsequent pond culture and stocking programs will require techniques for separating the hybrids from the parent species. Forty fingerlings (28-36 days old) of each group were measured to provide standard length (SL), body depth (BD), head length (HL), snout length (SN), eye diameter (EY), interorbital width (IO), maxillary length (MX), and pelvic fin length (PL). Body proportions of BD:SL, HL:SL, SN:HL, EY:HL, IO:HL, MX:HL, and PL:HL were calculated. The hybrid BD:SL, SN:HL, and PL:HL ratios were intermediate between the parents, whereas hybrid HL:SL and EY:HL ratios were greater than the parents. A series of two-phase discriminant analyses were preformed on the body ratios to determine if the morphometric ratios can reliably distinguish between parents and hybrids. The initial analysis yielded a discriminant function which correctly classified 95.0% of the fish. Subsequently, additional fish from each group were measured by independent observers to validate the original function. The second analysis resulted in 75.0% of the fish being correctly classified. All data were used to provide a new discriminant function which correctly classified 90.8% of the fish. Therefore, morphometric analysis can be used to distinguish between the parents and both hybrids where fingerlings are stocked together.

INTRODUCTION

Spotted seatrout *Cynoscion nebulosus* (SST) support an important recreational fishery along the southeastern Atlantic and Gulf coasts (Perret, Weaver, Williams, Johansen, McIlwain, Raulerson and Tatum 1980). Similarly, the orangemouth corvina *C. xanthulus* (OMC) is a valued game fish in the Gulf of California and the Salton Sea (Whitney 1961, Black 1974). Successful artificial propagation of these fishes has allowed hatchery production for stocking programs and stimulated interest in intrageneric hybridization of both species. An SST female × OMC male hybrid and the reciprocal OMC female × SST male hybrid were produced between 1984 and 1986, and have been

cultured and pond reared (Maciorowski, Colura, Henderson-Arzapalo and Bumguardner 1986). The two hybrids and the OMC have all been stocked into inland reservoirs in Texas (Texas Parks and Wildlife Department, unpublished data).

Fingerlings produced in hybridization trials have not been morphologically described nor compared with the parent species. This investigation describes morphological differences between the parent species and hybrids, and presents morphometric characteristics useful in separating hybrids from the two parent species. Such information is useful in fish population surveys conducted where the hybrids and OMC have been stocked together. A series of discriminant function analyses (Klecka 1980) were utilized to produce models for distinguishing between the four groups of fish.

MATERIALS AND METHODS

Spotted seatrout, orangemouth corvina, spotted seatrout female × orangemouth corvina male hybrid (SST × OMC), and orangemouth corvina female × spotted seatrout male hybrid (OMC × SST) fingerlings were produced by spawning procedures and pond culture methods described by Maciorowski *et al.* (1986). Fingerling ages, harvest dates, and mean standard lengths and weights of 100 randomly selected fish from each group are presented in Table 1. All fingerlings were preserved in 4% buffered formalin until analysis.

TABLE 1

Harvest dates, age, mean (± SD) weights, and mean (± SD) standard lengths of fingerlings (n = 100) used in the morphometric analysis.

Species	Date harvested	Age (days)	Mean (±SD) weight (g)	Mean (±SD) standard length (mm)
Spotted seatrout	26 Aug 1985	32	0.69 ± 0.08	37 ± 2.0
Spotted seatrout X orangemouth corvina	18 Jan 1985	33	1.14 ± 0.29	41 ± 4.1
Orangemouth corvina X spotted seatrout	25 Jul 1986	28	2.70 ± 0.40	53 ± 3.0
Orangemouth corvina	24 Sep 1986	36	1.00 ± 0.13	51 ± 1.2[a]

[a] N = 60

Body depth (BD), head length (HL), snout length (SN), eye diameter (EY), interorbital width (IO), maxillary length (MX), and pelvic fin length (PL), of 40 fish from each group (which were randomly selected from 100 preserved fish) were measured to the nearest 0.1 mm with an ocular micrometer and stereomicroscope. Standard length (SL) was measured to the nearest 1.0 mm using a caliper and steel rule. Arithmetically calculated body proportions included BD:SL, HL:SL, SN:HL, EY:HL, IO:HL, MX:HL, and PL:HL (Hubbs and Lagler 1958). Means, standard deviations (SD), and ranges of the body proportions were calculated for each group and compared by one-way analysis of variance and the Student-Newman-Keuls multiple range test ($\alpha = 0.05$).

A series of two-phase discriminant analyses (Klecka 1980) were performed to evaluate the above morphometric ratios as distinguishing characteristics between the parent species and hybrids (SAS Institute 1985; SPSS/ Inc. 1986). The two-phase discriminant analysis consists of two parts. A stepwise procedure permits only those variables contributing significantly to the separation of the groups to enter into the analysis. The subsequent classification procedure calculates discriminant functions which maximize separation between parent species and the two hybrids.

A V1 validation procedure (Ryder 1978) was performed on measurements generated by independent observers to quantify possible sampling error and search bias. Twenty fish from each group were measured and classified using the discriminant functions generated in the initial analysis. Finally, data from the initial measurements were combined with measurements from the independent observers to generate a new discrimination model based on all 240 fish.

RESULTS AND DISCUSSION

Morphometric body proportions and two-phase discriminant analysis can be used to distinguish between spotted seatrout, orangemouth corvina, SST × OMC hybrid, and OMC × SST hybrid fingerlings. With the exception of MX:HL, morphometric characteristics of the two hybrids were either intermediate to or larger than the parent species (Table 2). Mean BD:SL and PL:HL ratios were significantly different among all four groups, with values for both hybrids intermediate to those of the parents. The SN:HL ratio of the SST × OMC hybrid was intermediate to the parents, but not significantly different for the spotted seatrout and the OMC × SST hybrid. Hybrid HL:SL and EY:HL ratios were significantly larger than the parents. The IO:HL ratio was significantly smaller for orangemouth corvina than for the other 3 groups. Proportional measurements of spotted seatrout determined in this study were similar to those reported by Johnson (1978). Similar comparative data for orangemouth corvina and the two hybrids are not available in the literature.

Stepwise discriminant analysis indicated all seven calculated body ratios met criteria for entry into the model. Based on the magnitude of Wilk's lambda statistic, EY:HL, BD:SL, HL:SL, IO:HL, PL:HL, MX:HL, and SN:HL contributed to the separation of the four taxonomic groups in descending order of importance. The three discriminant functions (Wilk's $\lambda = 0.5189$) generated by the initial classification analysis correctly classified 95.0% of the specimens into the four groups. Discriminant functions 1 (DF1) and 2 (DF2) accounted for 61.6% and 27.9% of the variance among groups, respectively. The magnitude of the standardized canonical discriminant function coefficients (Table 3) used in the formation of the discriminant functions indicates the relative importance of each variable to the discrimin-

TABLE 2

Mean, standard deviations and range ( ) of body proportions (n = 40) for spotted seatrout, orangemouth corvina, spotted seatrout female × orangemouth corvina male, and orangemouth corvina female × spotted seatrout male. Within each body proportion, superscript indicates results of Student-Newman-Keuls ($P < 0.05$) multiple comparison tests.

	BD:SL	HL:SL	SN:HL	EY:HL	IO:HL	MX:HL	PL:HL
Spotted seatrout	^{1}4.28 ± 0.12 (4.1-4.5)	^{1}2.80 ± 0.09 (2.6-3.0)	^{1}3.87 ±0.25 (3.3-4.4)	^{1}4.94 ± 0.18 (4.6-5.5)	^{1}4.87 ± 0.39 (4.2-5.6)	^{1}2.35 ± 0.10 (2.2-2.6)	^{1}2.13 ± 0.3 (1.8-2.4)
Spotted seatrout X orangemouth corvina	^{2}4.08 ± 0.19 (3.8-4.8)	^{2}2.99 ± 0.10 (2.8-3.1)	^{2}4.08 ± 0.23 (3.6-4.6)	^{2}5.09 ±0.26 (4.5-5.5)	^{1}4.89 ±0.28 (4.3-5.7)	^{1}2.34 ± 0.12 (2.1-2.7)	^{2}2.00 ±0.10 (1.9-2.2)
Orangemouth corvina X spotted seatrout	^{3}4.14 ± 0.11 (4.0-4.4)	^{2}2.97 ± 0.10 (2.8-3.2)	^{1}3.90 ± 0.25 (3.3-4.5)	^{3}5.83 ± 0.22 (5.4-6.3)	^{1}4.88 ± 0.27 (4.4-5.9)	^{2}2.49 ± 0.11 (2.3-2.8)	^{3}1.94 ± 0.08 (1.8-2.1)
Orangemouth corvina	^{4}3.84 ± 0.09 (3.6-4.1)	^{1}2.84 ± 0.07 (2.7-3.0)	^{3}4.26 ±0.22 (3.8-4.9)	^{1}4.94 ±0.24 (4.4-5.6)	^{2}4.23 ±0.23 (3.9-4.7)	^{3}2.26 ± 0.09 (2.1-2.4)	^{4}1.85 ±0.09 (1.7-2.1)

* BD = body depth; SL = standard length; HL = head length; SN = snout length; EY = eye diameter; IO = interorbital width; MX = maxillary length; PL = pelvic fin length

TABLE 3

Standardized canonical discriminant function coefficients generated from the initial data set. Coefficient magnitudes indicate the relative importance of the variable to the discriminant function.

Body proportion[a]	Discriminant function 1	Discriminant function 2	Discriminant function 3
BD:SL	0.05527	0.63582	0.64191
HL:SL	0.69660	0.00244	0.90161
SN:HL	0.08862	0.22706	0.08619
EY:HL	0.85400	0.12875	0.43507
IO:HL	0.14090	0.23827	0.65948
MX:HL	0.37035	0.05865	0.00371
PL:HL	0.22274	0.47049	0.38618

[a] BD = body depth; SL = standard length; HL = head length; SN = snout length; EY = eye diameter; IO = interorbital width; MX = maxillary length; PL = pelvic fin length

ant function. (Table 4) indicates that EY:HL and MX:HL contribute considerably to DF1; BD:SL, PL:HL, IO:HL, and SN:HL contribute to DF2; and HL:SL contributes to DF3.

Incorrectly classified fish did not follow a recognizable pattern (*e.g.*, hybrids were not consistently misclassified as the reciprocal hybrid or either parent). Two SST were misclassified as the reciprocal hybrid or either parent). Two SST were misclassified as SST × OMC hybrids, two OMC were misclassified as either SST or SST × OMC hybrids, three SST × OMC were misclassified as either SST or OMC × SST hybrids, and one OMC × SST hybrid was misclassified as a SST × OMC hybrid. Four group discriminant analysis has a 25.0% prior probability of correct classification.

TABLE 4

Structure matrix of the correlations between the discriminating variables and the discriminant functions. Asterisk indicates variables which contribute to each function.

Body proportion[a]	Discriminant function 1	Discriminant function 2	Discriminant function 3
EY:HL	0.69845*	0.02440	0.39476
MX:HL	0.30793*	0.18241	0.19063
BD:SL	0.11664	0.77974*	0.14981
PL:HL	0.09178	0.64825*	0.03693
IO:HL	0.20973	0.51229*	0.26649
SN:HL	0.12248	0.37238*	0.20353
HL:SL	0.31896	0.07265	0.58099*

[a] BD = body depth; SL = standard length; HL = head length; SN = snout length; EY = eye diameter; IO = interorbital width; MX = maxillary length; PL = pelvic fin length

Data generated by independent observers were subsequently classified using discriminant functions developed from the initial observations. The four groups of fish were correctly classified with 75.0% accuracy. In the second analysis, 55.0% of the spotted seatrout were correctly identified; misclassified observations were all classified as SST × OMC hybrids. Orangemouth corvina, however, were correctly classified with 95.0% accuracy. SST × OMC and OMC × SST hybrids were classified with 70.0% and 80.0% accuracy, respectively, and were misclassified as each other more frequently than either of the parent species. The higher classification rate error was probably due to small sample sizes and inexperience on the part of the independent observers regarding the measurement of fish.

The initial measurements and data generated from the independent observers were combined to yield three new classification functions. As with the initial analysis, all seven body proportions met criteria for entry into the discriminant analysis. The three modified discriminant functions (Wilk's λ = 0.6708) correctly classified the combined data with 90.8% accuracy. The magnitude of the group separation shown graphically in Figure 1, is based on DF1 and DF2, which accounted for 55.2% and 36.0% of the variance among groups, respectively. SST, OMC, SST × OMC hybrids, and OMC × SST hybrids are classified as groups 1 through 4, respectively. The highest misclassification rate (15%) occurred for the SST × OMC hybrids, with most misclassifications placed in the OMC × SST hybrid group. The body proportions EY:HL and MX:HL contributed heavily to DF1, BD:SL, PL:HL, and IO:HL contributed to DF2, and HL:SL and SN:HL contributed to DF3.

The classification function coefficients generated from the combined data set (Table 5) are the most appropriate functions for field classification of the

TABLE 5

Classification function coefficients generated from the combined data set. Proportional measurements from an unclassified sample are entered in each equation[a], and the function yielding the highest discriminant score (D) indicates fish group.

Body proportion[b]	Spotted seatrout	Orangemouth corvina	SST X OMC	OMC X SST
BD:SL	98.665	84.323	89.326	94.103
HL:SL	404.708	197.261	424.881	424.640
SN:HL	67.931	70.340	70.006	67.842
EY:HL	74.628	77.659	79.751	89.992
IO:HL	15.956	12.955	15.340	13.463
MX:HL	138.428	134.085	151.528	144.158
PL:HL	225.543	210.217	222.094	211.114
(constant)	-1535.666	-1427.364	-1586.404	-1628.596

[a] For example, D = −1535.67 + 98.67 (BD:SL) + 404.71 (HL:SL) + 67.93 (SN:HL) + 74.63 (EY:HL) + 15.96 (IO:HL) + 138.43 (MX:HL) + 225.54 (PL:HL)

[b] BD = body depth; SL = standard length; HL = head length; SN = snout length; EY = eye diameter; IO = interorbital width; MX = maxillary length; PL = pelvic fin length

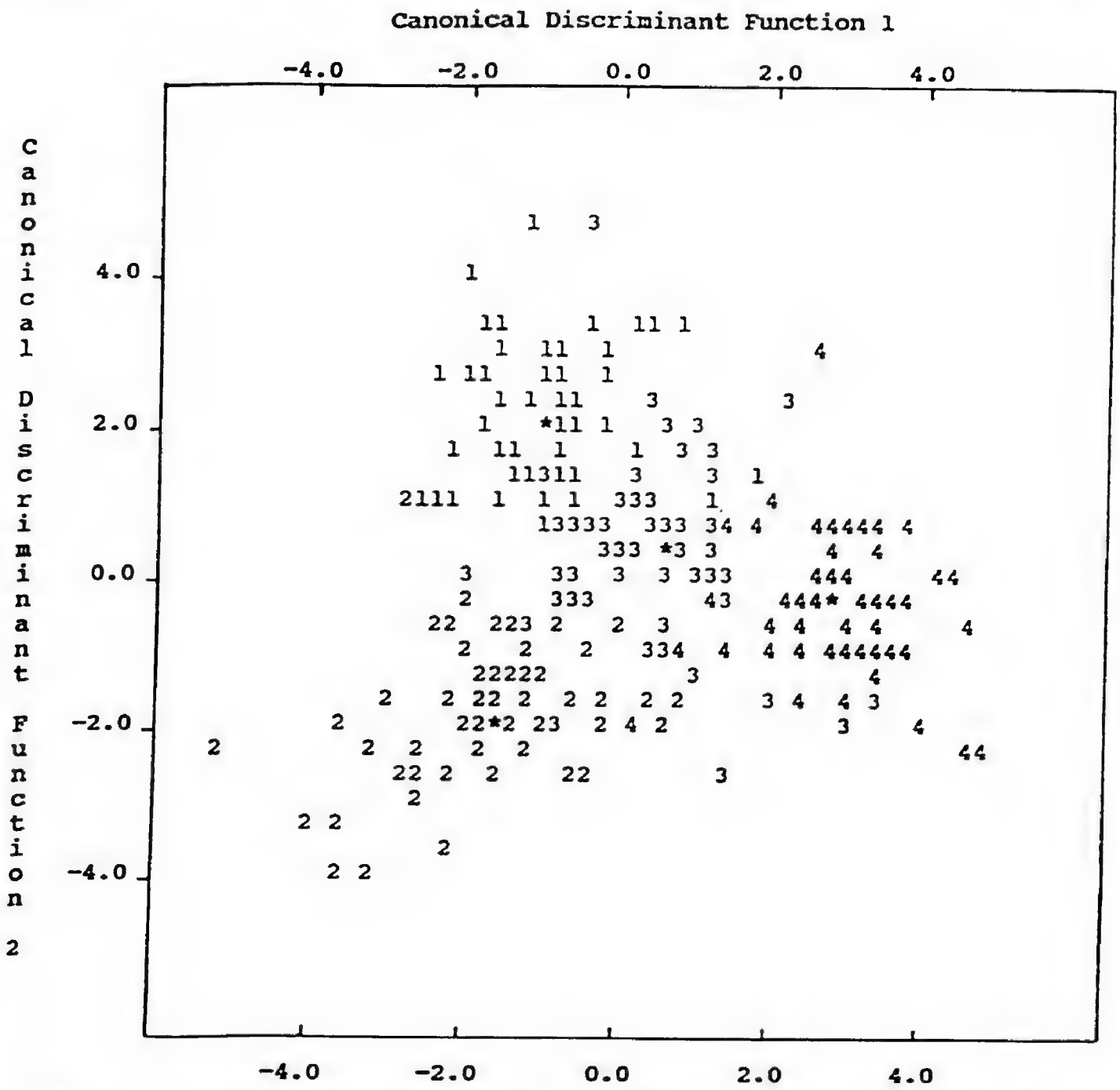

FIG. 1. Scatter plot indicating magnitude of group separation, based on discriminant functions 1 and 2 (1 = SST, 2 = OMC, 3 = SST × OMC, 4 = OMC × SST). Asterisk (*) indicates a group mean.

fish due to increased accuracy from the larger sample size. Body proportion ratios from a specimen are entered into each classification function, and the function which yields the highest discriminant score (D) indicates the fish group. We acknowledge that body proportions will change with age, therefore, comparable age groups are an essential element of this type of comparison. We do not intend to infer that the classification functions obtained in this study can be used to discriminate between adult fish. However, the minor age differences between groups of fish used in this study should not bias the results of these classification function coefficients.

Morphometric body proportions and two-phase discriminant analysis can be used to distinguish between spotted seatrout, orangemouth corvina, SST × OMC hybrids, and OMC × SST hybrid fingerlings with 90.8% accuracy.

This information may be useful in fish population surveys conducted where these fish have been stocked together.

ACKNOWLEDGMENTS

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GROWTH OF JUVENILE RED DRUM: ADENYLATE METABOLISM, RNA-DNA RATIO AND EFFECTS OF OVINE GROWTH HORMONE

Peter Thomas, Mark E. Westerman, P.F. Dehn[1], E. Nowicki, G. Jean Holt and C.R. Arnold

The University of Texas at Austin
Marine Science Institute
Port Aransas, Texas 78373

ABSTRACT

There is a paucity of information on the growth of cultured red drum. In the present study the growth rate of 360 juvenile red drum fed a commercial pelleted diet was monitored over an eight-week period. Mean body weight increased rapidly from 12.1 g to 89.5 g during the course of the experiment, with an average weight gain of 26% per week. This rapid growth rate was not further augmented by weekly injections of ovine growth hormone (1 or 5 μg/g body weight). However, in a preliminary 2-week trial biweekly injections with a high dose of ovine growth hormone (25 μg/g body weight) increased the growth rate 25% above that of controls. Techniques were developed to measure adenylate energy metabolism in juvenile red drum. Rapid preservation of muscle tissue by freeze-clamping was necessary to prevent postmortem changes in the adenylate pool. ATP levels in red drum dorsal epaxial white muscle were only 16-33% of the concentrations previously reported in other marine fish. Further studies will be required to determine whether these low levels of ATP reflect increased energy demands associated with rapid growth. RNA and DNA concentrations were also measured in white muscle. The RNA-DNA ratios did not differ among the three treatment groups. A parallel and significant increase in the RNA-DNA ratios of all treatment groups was observed during the third week of the study, the precise significance of which remans to be elucidated.

INTRODUCTION

Despite growing interest in the development of a red drum mariculture industry in Texas and other Gulf coast states, many aspects of the rearing of this species in captivity remain poorly understood. For example, precise information on the growth of cultured red drum is currently lacking.

This paper reports the results of some initial studies on growth of juvenile red drum reared in raceways on a commercial fish diet. Red drum epaxial muscle RNA and DNA concentrations were monitored during these studies, since the RNA-DNA ratio has been proposed as a sensitive indicator of recent and potential somatic growth in fishes (Bulow 1987). Preliminary data were also obtained on muscle tissue reserves of adenylates and substrates of energy metabolism, which are consumed in large quantities during periods of protein synthesis and rapid growth (Brett and Groves 1979). In addition the

[1] The University of Texas at San Antonio, Life Science, San Antonio, Texas 78285-0662, USA

efficacy of a heterologous growth hormone in promoting growth of red drum was examined. Significant enhancement of growth has previously been reported in several cold-water species administered growth hormones (Donaldson, Fagerlund, Higgs and McBride 1979; Sekine, Mizukami, Nishi, Kuwana, Saito, Sato, Itoh and Kawauchi 1985; Degani and Gallagher 1985).

METHODS

Approximately 750 juvenile red drum (length 8.92 ± 0.55 cm, weight 12.51 ± 1.74 g, $\bar{x}$ ± S.D.), which had been reared on a commercial trout diet (Purina Trout Chow, No. 2, 1.6 mm and No. 3, 2.4 mm), were divided equally among six 1200 L-raceways (two replicates of three treatment groups). The raceways were supplied with running seawater (12.25 L min; temperature 28-30°C, salinity 32-36‰ S). Fish were exposed to a long photoperiod (14:10, L:D) and were fed a commercial trout diet (Purina Trout Chow, No. 3, 2.4 mm and No. 4, 3.2 mm, containing 53% crude protein, 12% crude fat, 5% crude fiber) to satiation three times daily. During the course of the eight-week study the amount of food added daily to the raceways declined from 10% to 3.5% of fish body weight (approximately 80% of the food was consumed). Once a week the fish were anesthetized with MS-222 (80 mg L), weighed to the nearest 0.1 g, their standard lengths recorded, and then injected with 1 or 5 μg ovine growth hormone per gram body weight (b.w.) or with saline (controls). Ovine growth hormone (oGH, lot no. AFP 7649C, NIH) was dissolved in 0.9% NaCl (pH 10-10.5) immediately before use and injected intramuscularly into the right epaxial muscle (1 μL g body weight fish). In a second, two-week study, red drum were injected with either a high dose of oGH (25 μg g b.w.), or saline twice weekly (two raceways per treatment). At regular intervals whole fish were rapidly frozen on dry ice and stored at −80°C for up to 10 days until muscles tissues were analyzed for RNA and DNA content. Approximately 15 mg of the epaxial white muscle tissue was excised and homogenized in 0.8 ml ice-cold 1M NaCl. Homogenates were centrifuged at 3,000 × g for 20 min at 4°C and duplicate 100 μL aliquots of the resulting supernatants were analyzed for RNA and DNA by a modification of the fluorometric procedure of Bentle, Dutta and Metcoff (1981).

Two methods of preserving red drum tissues for analysis of adenylates and substrates of energy metabolism were compared: freezing the fish whole on dry ice and rapid excision of the muscle followed by freeze-clamping in liquid nitrogen. Frozen muscle was ground in liquid N^2 and extracted with perchloric acid (Dehn, Haya and Aiken 1985). The concentrations of the adenylates, phosphocreatine, glucose and glycogen in the neutralized extract were determined spectrophotometrically using modifications of the enzymatic methods of Jaworek, Gruber and Bergmeyer (1974), Keppler and Decker (1974), Lamprecht and Trautschold (1974) and Lamprecht, Stein, Heinz and Weisser (1974). Tris buffer (pH 7.6, 50 mM) was substituted for triethanolamine buffer and the assay volumes adjusted for measurement in red drum muscle. Glycogen was calculated from the difference in glycosyl units before and after amyloglucosidase hydrolysis. Total adenylate pool (TA) was also calculated.

RESULTS AND DISCUSSION

Growth was rapid in juvenile red drum fed the commercial diet (Fig. 1a, 1b). Mean body weight increased from 12.1 g to 89.5 g during the eight-week study, equivalent to an average weight gain of 26% per week or 1.38 g per day. Body length increased from a mean of 8.7 cm to 17.6 cm during this period, with a mean increase of 9.2% per week. Fish grew an average of 1.6 mm per day, about three times the growth rate of their wild counterparts (Reagan 1985).

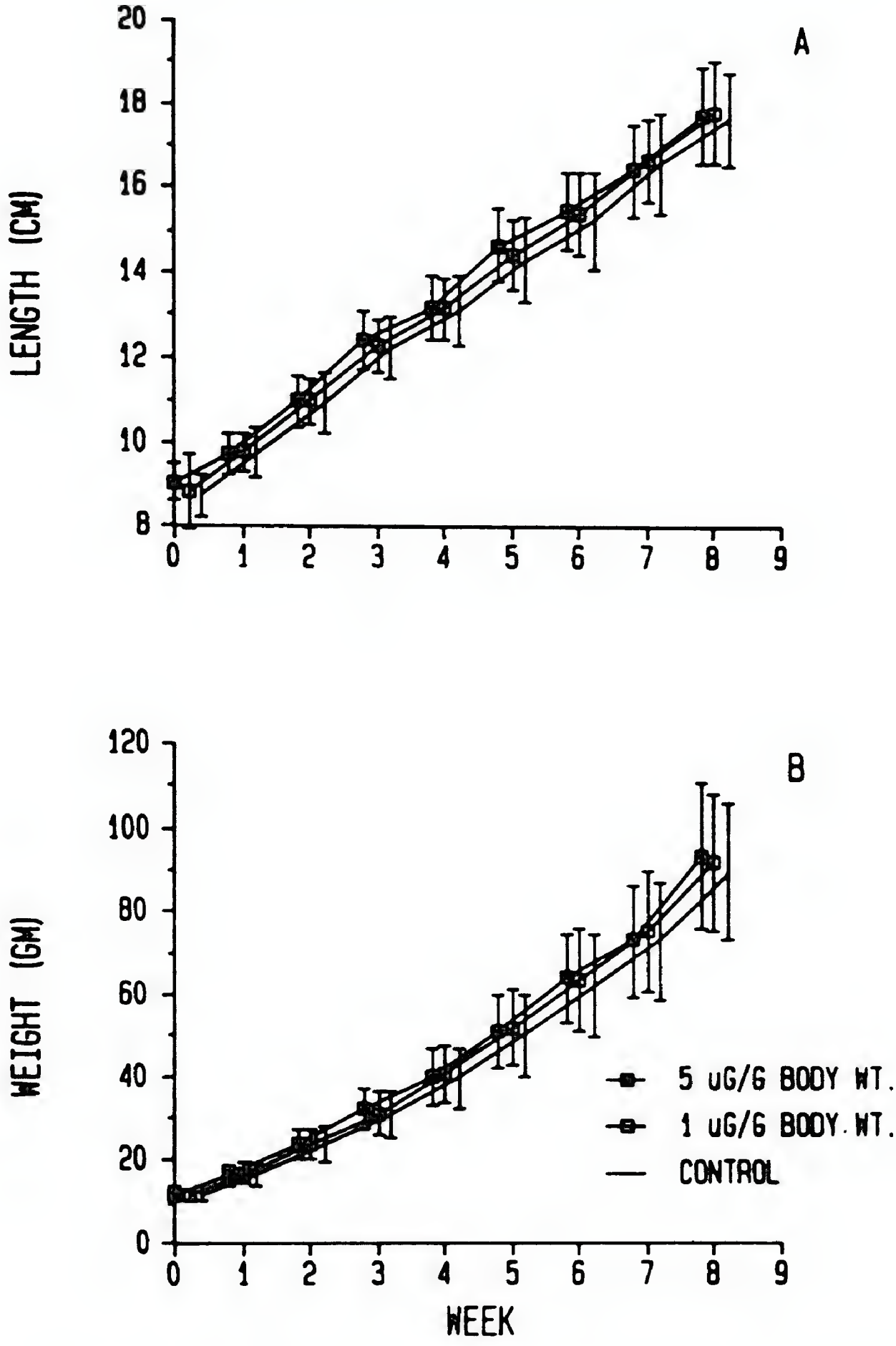

FIG. 1. Growth of juvenile red drum (*Sciaenops ocellatus*) reared in a raceway on a commercial pelleted diet over an eight-week period. Fish were injected with 0, 1 or 5 μg ovine growth hormone per gram body weight once a week. Each point represents the mean ± standard deviation of 80-125 measurements:

A. Increase in body length (total length),

B. Increase in body weight.

Growth of red drum was not enhanced by weekly injections of 1 or 5 μg ovine growth hormone per gram body weight (Fig. 1a, b, although these dosing regimens were effective in promoting growth of several cold water species (Donaldson, *et al.* 1979; Sekine, *et al.* 1985). However, biweekly injections with 25 μg ovine growth hormone per gram body weight for two weeks increased weight gain 25% above that of controls (Fig. 2). The

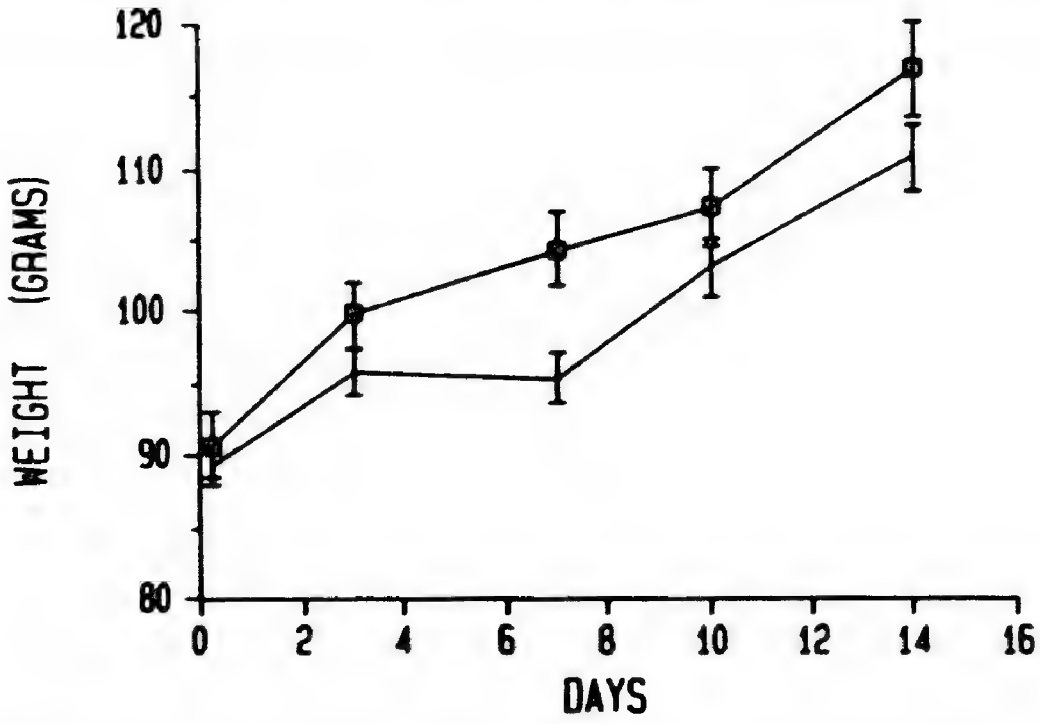

FIG. 2. Effect of twice-weekly injections of a high dose of ovine growth hormone (25 μg/g body weight) on body weight of juvenile red drum, (*Sciaenops ocellatus*). Each point represents the mean ± standard deviation of 21 measurements. Open squares - hormone-treated group.

growth promoting effect of this high dose of ovine growth hormone remains to be examined over a longer period of treatment. However, these preliminary results suggest that mammalian growth hormones have the potential for enhancing the growth of cultured red drum. The addition of mammalian growth hormones in the diet has been shown to promote growth in several fish species (Donaldson *et al.* 1979; Degani and Gallagher, 1985). Currently the high cost of these hormones precludes their use as additives to commercial fish feeds. However, the recent development of techniques to produce large quantities of recombinant growth hormones may make the addition of growth hormones to fish diets economically feasible.

Analysis of nucleic acid concentrations in muscle tissues of red drum revealed no significant differences in the concentration of RNA or DNA per milligram tissue or the RNA-DNA ratio between controls and both growth hormone treatment groups (1 and 5 μg/g b.w., Fig. 3) within each of the three weeks tested. However, there was a significant increase in the RNA-DNA ratio in all treatment groups between the second and third weeks (Fig. 3). This increase was due to an increase in RNA concentration (week two $\bar{x}$ all teatments 2.72 ± 0.37 μg/mg; week three $\bar{x}$ all treatments 3.11 ± 0.43 μg/mg; $P < .003$) and a decrease in DNA/mg muscle tissue (week two $\bar{x}$ all treatments

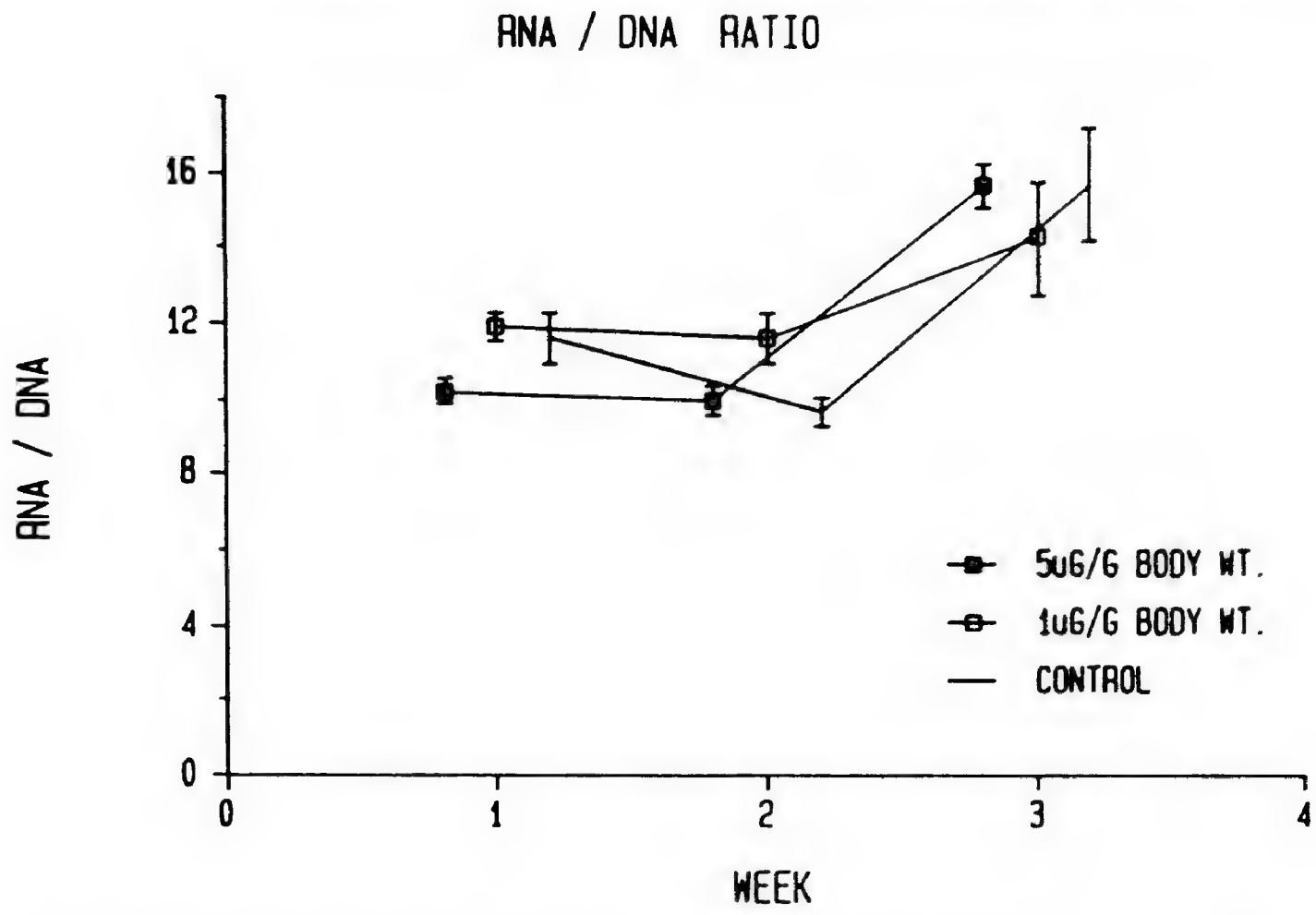

FIG. 3. The RNA-DNA ratios of red drum juveniles injected weekly with 0, 1, or 5 μ/g body weight ovine growth hormone. Each point represents the mean ± S.E. of 10 measurements.

0.26 ± 0.03 μg/mg; week three $\bar{x}$ all treatments 0.21 ± 0.04 μg/ mg; $P < .0001$). These data indicate a period of hypertrophic growth which is characterized by an increase in cell size (Goss, 1966; Bulow, 1987).

The concentrations of RNA and DNA per milligram tissue or per milligram protein have been used extensively in mammals and to a lesser extent in fish (Love, 1970) as indices of nutritional condition, protein synthesis, and type of cellular growth (Waterlow, Garlick, and Melward, 1978). Kayes (1979) observed significant increases in the RNA-DNA ratio in both muscle and liver tissue of black bullhead *Ictalurus melas* administered bovine growth hormone compared to hypophysectomized controls. Nucleic acid concentrations and the RNA-DNA ratio could prove to be valuable tools for investigating growth patterns in cultured marine finfish. However, further studies of the relationships among somatic growth and the tissue concentrations of RNA, DNA and their ratio are necessary to determine whether these indices are useful for monitoring growth in fish.

Freeze-clamping in liquid nitrogen resulted in significantly higher increases in the concentrations of all the metabolites compared to the values obtained after freezing on dry ice (Table 1). These results indicate that rapid preservation of the tissue is necessary to prevent post-mortem changes in the adenylate and energy precursor pools. Few studies have examined adenylates or substrates of energy metabolism in fish during growth or during the

TABLE 1

Effects of preservation method on concentrations of adenylates and substrates of energy metobolism in muscle

Parameter	Concentration (μ mol/g wet weight) frozen on dry ice	frozen in liquid nitrogen
Adenylates		
ATP	0.15 ± 0.04[1]	1.35 ± 0.20*
ADP	0.03 ± 0.01	0.65 ± 0.05*
AMP	0.03 ± 0.01	0.23 ± 0.03*
Total adenylates	0.21 ± 0.05	2.23 ± 0.23*
Energy substrates		
Phosphocreatine	0.11 ± 0.02	0.51 ± 0.07*
Glucose	0.67 ± 0.04	0.83 ± 0.08*
Glycogen	2.17 ± 0.57	9.27 ± 1.30*

[1] X ± S.E.M., n = 10

* P < .05, Student's t test

juvenile stage. Atlantic salmon juveniles had freeze-clamped muscle ATP levels that were five times, and energy precursor levels that were 0.1 to 11 times higher, than those of red drum freeze-clamped dorsal muscle (Haya, Waiwood and Van Eeckhaute 1985). It is not known whether the lower levels of energy molecules in the red drum juveniles reflect increased energy demands associated with the rapid growth of this warm-water species.

ACKNOWLEDGMENTS

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EVALUATION OF ETOMIDATE AS AN ANESTHETIC FOR RED DRUM, *SCIAENOPS OCELLATUS*

W. W. Falls, G. K. Vermeer, and C. W. Dennis

Florida Department of Natural Resources
Institute of Marine Research
100 Eighth Avenue S.E.
St. Petersburg, Florida 33701-5095

ABSTRACT

Etomidate was evaluated as an anesthetic in fourteen female and seven male red drum (mean standard length (SL) = 83.55 cm; mean weight = 9.57 kg). Fish were anesthetized every 2 or 3 weeks with etomidate dosage levels of 0.8, 1.6, or 8.0 mg/L during a pre-spawning conditioning regime. The 8 mg/L dosage was found to be too high as fish reached stage 4 anesthesia (loss of reflex activity) in less than 30 sec. At a 0.8 mg/L dosage, mean induction and recovery times were excessively long (6.5 and 10.22 min., respectively). Further trials at these 2 dosages were discontinued. Between the induction and recovery periods, fish were maintained in a respirator at a 0.4 mg etomidate/L dosage for a mean time of 17.47 min. At 1.6 mg/L, mean induction time was 4.06 min., and mean recovery time was 5.68 min. This dosage appeared suitable for adult red drum as anesthesia and recovery times were acceptable, fish showed normal behavior after recovery, began feeding the next day, and gonadal recrudescence continued normally in preparation for spawning. An induction dose of 1.6 mg/L and a respirator maintenance dose of 0.4 mg/L appeared suitable for routine fish handling.

INTRODUCTION

Etomidate is an experimental, non-barbiturate hypnotic, water-soluble anesthetic which has been tested intravenously in humans, mice, rats, guinea pigs, rabbits, and dogs (Janssen,Niemegueers and Marsboom 1975). Etomidate has been shown to be safer, more economical, and less stressful than quinaldine or tricane methane sulfonate (MS-222) when used in water as an anesthetic on golden shiner, striped bass, channel catfish, bluegill, zebra danio, black tetra, angelfish, and southern platyfish (Amend, Goven and Elliot 1982, Davis, Parker and Suttle 1982, Limsuwan, Grizzle and Plumb 1983, Plumb, Schwedler and Limsuwan 1983). Davis *et al.* (1982) determined that, in yearling striped bass, etomidate caused a smaller increase in plasma corticosteroids than either quinaldine or MS-222. Limsuwan *et al.* (1983) noted that fish (channel catfish, golden shiner, and bluegill) anesthetized with 6-10 mg/L etomidate, lost reflex responses that could interfere with gamete stripping, surgery or blood collection.

Metomidate (an analog of etomidate) has been compared with MS-222 and quinaldine in red drum (Thomas and Robertson, unpublished report). These

researchers determined that metomidate exposure (7 mg/L) did not increase plasma cortisol levels and induce glucose stress as did exposure to either quinaldine or MS-222.

The objective of this study was to evaluate etomidate as an anesthetic used during routine biopsy, blood collection and gamete stripping procedures in conditioning red drum.

MATERIALS AND METHODS

Twelve female and nine male red drum, collected from the East coast of Florida, were randomly divided into three equal groups. Each group of seven fish (four females and three males) was started on a 90-day, 120-day, or ambient spawning conditioning cycle (Roberts, Harpster and Henderson 1978). The 120-day cycle fish were conditioned in 12,600 L circular fiberglass tanks equipped with biological, diatomaceous earth, and settling filters (Roberts *et al.* 1978). The 90-day cycle fish were conditioned in a 18,000 L tank of sandfiltered natural seawater from Bayboro Harbor. Artificial seawater for the 90 and 120-day conditioning tanks was made from aged tap water and Instant Ocean synthetic seasalts. Tank water was sampled twice weekly for ammonia, nitrite, and nitrates. Oxygen and temperature were checked daily. Partial water exchanges were carried out as needed. All three groups were fed to satiation (approximately 5% body wt) five times/week with frozen shrimp, squid, and cigar minnows. Fish were not fed on sampling days.

Fish to be sampled for oocytes and blood were netted from the conditioning tank and placed in a 50 gal (189.3 L) rectangular induction tank containing aerated water from the conditioning tank and the appropriate dosage of etomidate. The induction time was the time in anesthetic until caudal peduncle motion ceased (Stage 3, Level 2) (Schoettger and Julin 1967). Fish were then removed from the induction tank, weighed, measured, and placed in a respirator, which pumped a maintenance dose of 0.4 mg/L etomidate across the gills. While on the respirator, approximately 2 ml blood was collected by sterile syringe from the gill arch or sinus venosus. A catherized ovarian biopsy was taken from female fish, and males were palpated to determine if milt was flowing. The respirator time was the time from placement of the fish on the respirator to their return to the culture tank. After sampling, fish were returned to the conditioning tank and time to return of equilibrium (recovery time) was recorded. Fish were examined on days 1, 21, 42, 63, 98, and 112.

A separate group of 18 red drum from the Florida west coast was anesthetized using the same procedure described above and tagged with an internal anchor tag. These fish were only anesthetized once, at the start of a 120-day conditioning cycle.

RESULTS AND DISCUSSION

Etomidate dosage trials were performed on red drum broodstock using 0.8, 1.6 and 8.0 mg/L dosages. Two fish exposed to the 8.0 mg/L etomidate reached Stage 4 anesthesia (loss of reflex activity) (Schoettger and Julin 1967) in less than 30 sec and had excessively long recovery times (> 60 min). A dosage level of 0.8 mg/L resulted in mean induction and recovery times of 6.5 and 10.22 min, respectively. A 6.5 min induction time is considered too long by most fishery workers (Marking and Meyer 1985). It is not known why recovery times were so long at the lowest dosage which is contrary to what was expected. Further trials at both these dosages were discontinued.

Data for 4-2-87 (Table 1) refer to West Florida coast red drum. Data from these fish were analyzed separately due to differences in size and sampling

TABLE 1

Number of fish/trial; mean standard lengths; mean weights; and mean induction, respirator, and recovery times for seven sampling periods using 1.6 mg etomidate/L on red drum

Sampling Date	No. Fish Tested	SL(cm)	Wt(Kg)	Induction Time Min (mean S.E.)	Respirator Time Min (mean S.E.)	Recovery Time Min (mean S.E.)
East coast Red Drum						
3-10-87	20	83.55	9.57	3.92 (±0.14)	15.48 (±6.55)	5.26 (±2.94)
3-31-87	21	84.33	9.97	3.76 (±0.32)	11.15 (±4.19)	2.13 (±0.07)
4-21-87	21	83.05	10.16	2.15 (±0.16)	10.33 (±4.32)	3.54 (±1.52)
5-12-87	17	83.01	10.08	4.10 (±0.55)	12.19 (±3.54)	3.30 (±1.73)
5-26-87	18	83.68	10.34	2.58 (±0.13)	9.39 (±1.31)	5.31 (±1.39)
6-09-87	12	83.58	11.18	3.84 (±0.46)	9.38 (±4.82)	2.85 (±0.72)
West coast Red Drum						
4-02-87	18	54.73	3.18	5.21 (±1.22)	10.63 (±4.51)	3.42 (±1.16)

schedule. Data from the other six sampling dates are from East Florida coast red drum on 90-day, 120-day, or ambient conditioning cycles. Mean induction times for red drum from Florida's east coast for the 1.6 mg/L dosage ranged from 2.15 to 4.10 min. and mean recovery times ranged from 2.13 to 5.31 min. Induction time variances were homogenous between groups ($\chi^2 = 17.7$, $df = 108$, Bartlett's test for homogeneity of variances). Mean induction times were significantly different ($F = 1.70$, $df = 58,50$, $p < 0.05$) and the times from 4-21-87 and 5-26-87 were significantly less than the other dates (Studentized Maximum Modulus Test). Induction times were not significantly affected by variations in temperature (18.5-29.4°C), salinity (24-28‰), photoperiod (10.5-14.25 hours light/day), weight (9.57-11.18 kg), standard length (83.01-84.33 cm), or sex. Recovery time variances were quite heterogeneous ($\chi^2 = 411$, $df = 108$) which precluded examining mean effects with analysis of variance. There was, as expected, a significant difference between respirator times for males and females because ovarian biopsies were more time consuming than milt checks ($F = 14.0$, $df = 1,50$, $p < 0.05$).

A 0.4 mg/L respirator dosage maintained fish anesthesia for up to 23 min. Each fish was anesthetized up to six times during the 120-day conditioning cycle with no evidence of harm. Plumb *et al.* (1983) used etomidate induction dosages of 0.8 and 1.2 mg/L for two-year-old channel catfish. Davis *et al.* (1982) induced yearling striped bass with a dosage of 0.1 mg/L etomidate. Amend *et al.* (1982) found that for four species of aquarium fish the minimum effective dose ranged from 2.0 to 4.0 mg/L. In our study, etomidate at 1.6 mg/L appeared to be a safe and effective dosage for broodstock red drum. At this dosage, fish induced and recovered in an acceptable time, showed normal behavior after recovery, began feeding the next day, and gonadal recrudescence continued normally in preparation for spawning. We did not compare

etomidate to quinaldine and MS-222. Other researchers (Plumb *et al.* 1983), found that etomidate has several advantages over quinaldine and MS-222, *e.g.* lower effective concentration, possibility of longer exposure time, high water solubility, and absence of color or odor. We found etomidate at 1.6 mg/L to be a safe and effective anesthetic for broodstock red drum. Further evaluation of etomidate is needed to obtain U.S. Food and Drug Administration (F.D.A.) registration for its use on food fish.

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SECTION II

Reproduction and Genetics

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INDUCED SPAWNING OF SPOTTED SEATROUT, RED DRUM AND ORANGEMOUTH CORVINA (FAMILY: SCIAENIDAE) WITH LUTEINIZING HORMONE-RELEASING HORMONE ANALOG INJECTION

Peter Thomas and N. Boyd

The University of Texas at Austin
Marine Science Institute
Port Aransas, Texas 78373

ABSTRACT

The effect of a superactive analog of luteinizing hormone-releasing hormone ($LHRH_a$) on ovulation and spawning of three sciaenid fishes, spotted seatrout (*Cynoscion nebulosus*), red drum (*Sciaenops ocellatus*) and orangemouth corvina (*Cynoscion xanthulus*) with fully grown oocytes was investigated. Single injections of $LHRH_a$ (D-Ala^6, des-Gly^{10}-LHRH-ethylamide) at 0.1 mg/kg body weight resulted in successful spawning of all three species around dusk 30-35 hours post-injection. A second spawn occurred at dusk on the following day in all six trials. The results indicate that a single injection of $LHRH_a$ is a reliable method of inducing ovulation in three species of sciaenid fishes resulting in predictable spawning on the following day.

INTRODUCTION

Interest in the culture of sciaenid fishes has grown rapidly in the last five years. Manipulation of photoperiod and temperature has proven to be a reliable method of inducing spawning of red drum (Arnold, Bailey, Williams, Johnson and Lasswell 1977; Roberts, Harpster and Henderson 1978) and is now widely used in fish hatcheries to propagate this species. However, further refinement of artificial techniques for predictable induction of spawning is required for other sciaenid fishes of mariculture potential, such as spotted seatrout and orangemouth corvina (Prentice and Colura 1984).

Luteinizing hormone-releasing hormone analogs ($LHRH_a$), alone and in combination with dopamine antagonists, have been used successfully to spawn a variety of teleost species including carp (Cooperative Team for Hormonal Application in Pisciculture 1977), coho salmon (Van der Kraak, Lin, Donaldson, Dye and Hunter 1983), goldfish (Chang and Peter 1983), African catfish (de Leeuw, Resink, Rooyakkers and Goos 1985), milkfish (Marte, Sherwood, Crim and Harvey 1987) and mullet (Lee, Tamaru, Miyamoto and Kelley 1987). Thus, $LHRH_a$ has considerable potential as a practical tool for inducing ovulation in important pisciculture species (Donaldson and Hunter 1983).

Recently we reported the successful spawning of orangemouth corvina following two injections of $LHRH_a$ and pimozide (Prentice and Thomas 1987). The purpose of the present study was to determine the efficacy of $LHRH_a$ alone in inducing ovulation and spawning of three sciaenid fishes, spotted seatrout (*Cynoscion nebulosus*), red drum (*Sciaenops ocellatus*) and orangemouth corvina (*Cynoscion xanthulus*).

METHODS AND MATERIALS

Spotted Seatrout Experiments

Twelve adult spotted seatrout (33-51 cm TL) were collected by netting in March 1987 in the vicinity of Port Aransas, Texas and acclimated to laboratory conditions in a 16,000 L circular tank with an external recirculating biofilter and fed dead shrimp and squid at approximately 3% of their body weight per day. Salinity varied between 30 and 35‰. Photoperiod and water temperature were adjusted to mimic seasonal changes beginning with a photoperiod of 11L:13D and 17°C in March and progressing to 15L:9D and 29°C in August-September when the females appeared gravid and at least one male released milt when light abdominal pressure was applied. On August 24 the fish were anesthetized in 15 ppm quinaldine sulphate and ten fish (eight females and two males) were injected intramuscularly (im) below the dorsal fin with des-Gly^{10}-[D-Ala^6]-luteinizing hormone-releasing hormone ethylamide ($LHRH_a$) dissolved in acidified saline (pH 6) at a dose of 0.1 mg kg body weight. Two uninjected females served as controls. Nine of the $LHRH_a$-injected fish (seven females and two males) received a second injection of the hormone on September 16. Spawning was determined by the presence of eggs in a net placed under the outflow pipe from the tank.

Red Drum Experiments

Six adult red drum 74-90 cm TL were collected from a power plant cooling lake in the fall of 1986 and acclimated to a 16,000 L recirculating circular tank (salinity 30-35‰) described previously. Fish were fed beef liver, dead shrimp and dead squid at 3% of their body weight per day. In January 1987 fish were subjected to a condensed 150 day annual cycle as described by McCarty, Geiger, Sturner, Gregg and Rutledge (1986) beginning with late fall conditions (11L:13D and 19°C) to stimulate gonadal recrudescence. Female red drum were judged eligible for ovulation induction when the photoperiod and temperature reached fall conditions (11L:13D and 25°C) in September 1987 and their mean oocyte diameters reached 600 µm. Both males were spermiating at this time. However, none of the fish had spawned. Fish were anesthetized in 15 mg L quinaldine sulphate and intraovarian samples of oocytes were collected using a catheter inserted into the oviduct. Diameters of twenty oocytes from each individual were measured to the nearest 40 µm with an ocular micrometer at 25 × magnification. On September 12, 1987 the fish were anesthetized in quinaldine sulphate and four of the six fish (two females and two males) received injections of 0.1 mg $LHRH_a$/kg bw in saline im below the dorsal fin. The remaining two fish (one female and one male) were injected with saline and served as controls. On September 21, 1987, the two fish which had previously received only saline were injected with 0.1 mg $LHRH_a$/kg bw. Eggs were collected from the tank outflow.

Orangemouth Corvina Experiments

Orangemouth corvina were collected from the Salton Sea as subadults and reared to adult size on a condensed annual cycle as described by Prentice and Thomas (1987). Nine fish (four females and five males) were transferred to the Marine Science Institute in August 1986, and acclimated

to two 16,000 L tanks (Spring conditions: 12L:12D, 23°C, salinity 37-40‰) described previously. All fish were fed dead shrimp at approximately 3% of their body weight per day. Exposure to the condensed annual cycle was continued until December 1986 when conditions were equivalent to Fall (11:5L:12.5D, 24.5°C). At this time the females appeared gravid with fully grown oocytes (diameter 440-480 μm, obtained by intraovarian biopsy) and the males were spermiating. However, no spawning had occurred. On December 8, 1986, three out of five fish in one tank (one female and two males) received a single injection (im) of 0.1 mg $LHRH_a$/kg bw. The remaining fish (one female and one male) received injections of saline and served as controls. On December 16, two fish in the other tank (one male and one female) were administered $LHRH_a$ and the remaining two fish received saline injections. Eggs were collected as described previously.

RESULTS

All six attempts to spawn spotted seatrout, red drum and orangemouth corvina with fully grown oocytes by injection with 0.1 mg $LHRH_a$/kg bw were successful and resulted in the production of large numbers of fertilized eggs. Spawning occurred around dusk, 30-35 hours after the hormone injection in all three species (Table 1). Further, eggs were released on two consecutive evenings in all six trials with $LHRH_a$. A second injection of $LHRH_a$ three weeks later resulted in an additional successful spawn of spotted seatrout (Table 1).

TABLE 1

Summary of spawning data for spotted seatrout, red drum and orangemouth corvina after a single injection of 0.1 mg $LHRH_a$/kg bw[1].

Species	Date	No. of females	Total Length (cm)	Time (hrs)	Total eggs collected	Fertilized eggs % hatch
Spotted seatrout	8/24/87	8	33-51	30-35	13,231,000	99.0
Spotted seatrout	9/16/87	7	33-51	30-35	4,759,000	98.1
Red drum	9/12/87	1	90	30-35	3,530,000	98.9
Orangemouth corvina	12/8/86	1	58	30-35	174,900*	100

* Minimum number, underestimate due to sampling problems

[1] Results of second trials with red drum and orangemouth corvina not included (incomplete egg data).

Percent fertility could not be accurately determined because a large proportion of the unfertilized eggs of these three species tend to sink and do not appear in the tank outflow. Approximately 75% of the eggs collected in the outflow were fertile. The percentage of fertilized eggs which hatched, however, was greater than 92% in all spawns.

DISCUSSION

The results suggest that a single injection of $LHRH_a$ at a dose of 0.1 mg/kg bw is a reliable technique for inducing ovulation and spawning of these three

species of sciaenid fishes. In addition, the spawning time can be predicted within a few hours, since in six separate trials spawning occurred 30-35 hours after $LHRH_a$ administration. Treatment with $LHRH_a$ did not prevent spotted seatrout and red drum from spawning subsequently, either by $LHRH_a$ injection or naturally (results not shown). Thus, injection with $LHRH_a$ appears to be a practical method of spawning sciaenid fishes, especially when predictable spawning is required.

An interesting finding was that in all six trials spawns occurred on two consecutive days following $LHRH_a$ injection, whereas the interval between spawns varied considerably in a group of naturally spawning red drum broodstock (Arnold 1988). The two days of spawning observed after hormone administration may be due to a sustained elevation of plasma gonadotropin titers, as has been shown previously in coho salmon administered this superactive analog (Van der Kraak *et al.* 1983). The recent finding that plasma gonadotropin levels remain elevated in adult female Atlantic croaker (Family: Sciaenidae) for at least 24 hrs after a single injection of 0.1 mg $LHRH_a$/kg bw (Copeland and Thomas 1987) provides further evidence for a persistent action of the analog.

We previously reported the successful spawning of orangemouth corvina using a combined treatment of 0.13 mg $LHRH_a$/kg bw and a dopamine antagonist, pimozide at a dose of 10 mg/kg (Prentice and Thomas 1987). However, the present results show that at this dose $LHRH_a$ alone is effective in inducing ovulation in sciaenid fishes. Pimozide does not potentiate the effect of 0.1 mg $LHRH_a$/kg on gonadotropin secretion in Atlantic croaker (Copeland and Thomas, unpublished data), but the efficacy of this combined treatment remains to be investigated at low doses of $LHRH_a$. Currently studies are in progress to determine whether lower doses of $LHRH_a$, alone and in combination with dopamine antagonists, are effective in inducing ovulation in these species.

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POPULATION STRUCTURE AND GENETIC VARIATION IN RED DRUM

John M. Wakeman and Paul R. Ramsey

Department of Zoology
Louisiana Tech University
Ruston, Louisiana 71272

ABSTRACT

From analyses of sample compositions of over 500 red drum collected along the Gulf Coast of Texas, Louisiana, Mississippi and Florida, preliminary estimates of some important population parameters were obtained. Parameters of the von Bertalanffy growth equation, derived from the collection data, were used to construct yield per recruit curves as a function of fishing mortality. At current harvest rates, the estimated yield per recruit entering the fishery is about 0.4 kg.

A wide variety of structural and enzymatic proteins (40 loci) were examined to document the extent of subpopulation differentiation. Genetic variability of red drum was comparable with that reported for other sciaenid species, being slightly lower than the usual individual heterozygosities of about 5% reported for marine teleosts. Differentiation into subpopulations was only weakly evident (F_{st} = 0.019). The low genetic divergence in this species may be due to panmixia among adults. The low degree of polymorphisms in this species has implications in aquaculture with regard to the use of genetic markers and the monitoring of inbreeding.

INTRODUCTION

In recent years, managers of the red drum fishery have become increasingly concerned about the dangers of recruitment overharvesting. Attention has been drawn to the need for detailed biological data and a better understanding of population structure of this species in the Gulf of Mexico. Because fishing pressure on this species is likely to continue to increase, red drum aquaculture in marine hatcheries offers considerable potential as a means of stock enhancement in areas now being overexploited. Sound management of such programs requires reliable genetic information that can be applied in the use of genetic markers and the monitoring of the extent and effects of hatchery inbreeding.

METHODS

In this note, we summarize the results of analyses of red drum population structure in the Gulf of Mexico based on demographic and genetic data obtained from sample compositions of 537 red drum collected at 13 sites along the coasts of Texas, Louisiana, Mississippi and Florida. To minimize gear selectivity effects, a variety of methods were used in collecting fish from each sampling locality. Determination of weight, standard length, and sex preceded removal of tissue samples (heart, liver, muscle and eye) from each fish.

The analyses included a derivation of preliminary estimates of population parameters needed by fishery managers for yield-per-recruit consideration. Horizontal starch gel electrophoresis was used to screen 22 enzymes and 7 structural proteins (40 loci) for polymorphisms. The electrophoretic data were analyzed to document the extent of population subdivision in this species (Ramsey and Wakeman 1987).

RESULTS

Our collection data indicated relatively large red drum populations in coastal bays along the Gulf of Mexico with average densities in the order of 75-100 individuals per hectare (Wakeman and Ramsey 1985). Table 1 summarizes the estimated values for parameters of Beverton and Holt's (1966) yield equation derived from our length-at-age and age-frequency data for Louisiana populations and compares them with similar estimates derived by Matlock (1984) for Texas populations. Our estimated M/K value (0.64) for Louisiana red drum was used to generate yield curves (with t_c = 1,2 and 3) as a function of the instantaneous fishing mortality coefficient (F) (Fig. 1). The current estimated position of the Louisiana red drum fishery shows a yield of about 0.4 kg/recruit and indicates overfishing of this species. Such a situation could be remedied by reducing fishing effort, increasing the minimum size limit, or enhancing the stock by seeding selected areas with hatchery-raised juveniles. Our analysis indicates that implementation of a minimum catch size limit of 30 cm (SL) would result in an increase of yield to approximately 0.7 kg/recruit in Louisiana.

TABLE 1

Estimated values for parameters of the Beverton-Holt equation for red drum populations in Louisiana (Wakeman and Ramsey 1985) and Texas (Matlock 1984)

State	Growth Parameters		Mortality Parameters		
	W (g)	K	Z	F	M
Louisiana	10593	0.2877	1.833	1.650	0.183
Texas	13253	0.2950	1.608	1.190	0.420

Among the 29 proteins surveyed, six polymorphisms were detected — Adh-A (alcohol dehydrogenase), Aat-A (amino aspartate transferase), Gpi-B (glucose phosphate isomerase), G-3pdh-A (glycerol-3-phosphate dehydrogenase), Pgm-B (phosphoglucomutase), and transferrin. Variability in Icd-A (isocitrate dehydrogenase) has also been reported in two of seven Texas bays by Wilder (1986). Although genetic variation in red drum (H = 3%) is similar to other shore species (Waples 1987), the proportion of polymorphic loci is somewhat less than has been reported in other sciaenid species. Population differentiation (F_{st} = 0.019) appears to be similar to other sciaenids but lower than values generally reported for shore species (Table 2).

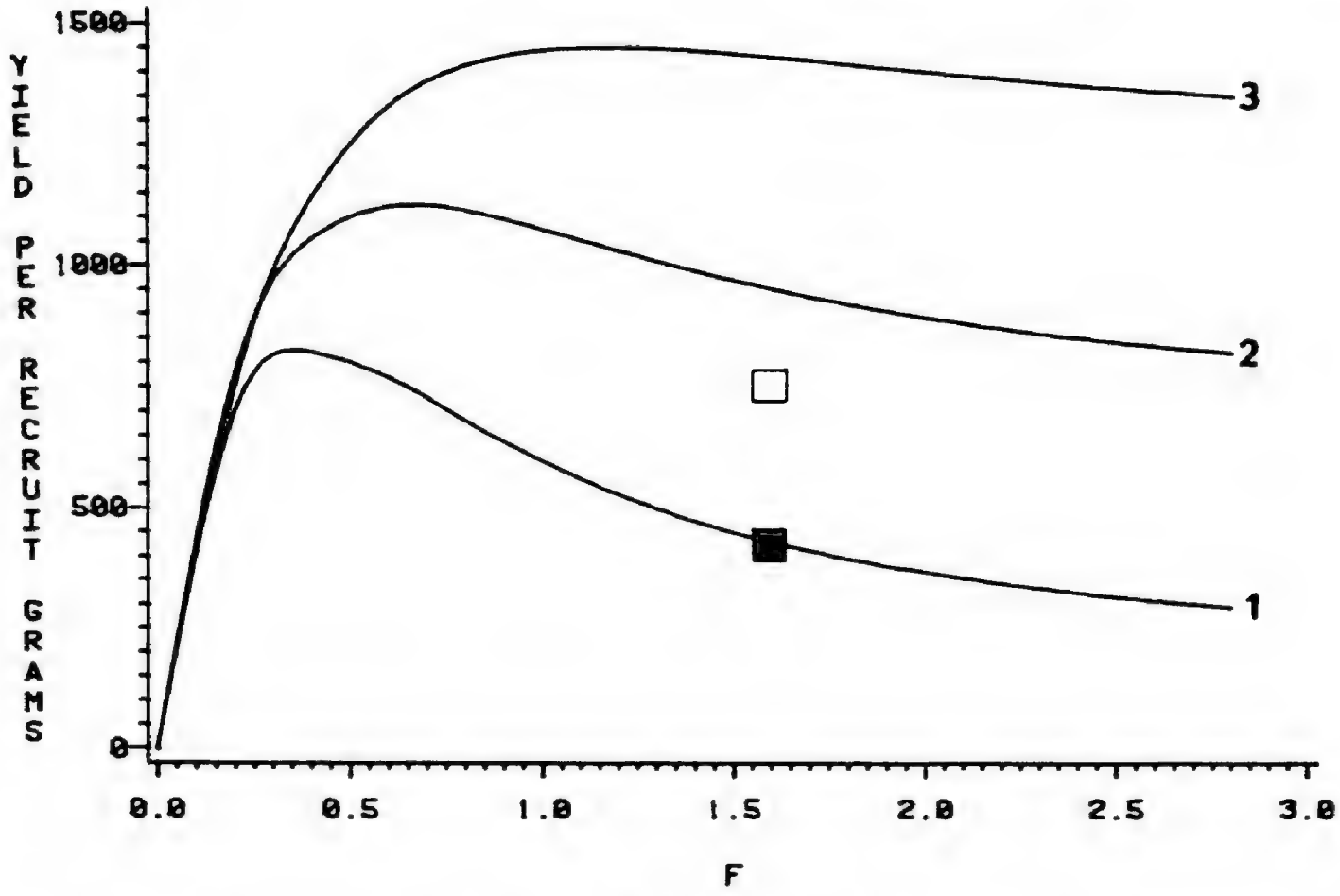

FIG. 1. Yield curves (with t_c = 1,2 and 3) as a function of F for red drum. The closed square indicates the current estimated position of the fishery in Louisiana and the open square indicates the estimated position if standard length at first capture were set at 30 cm.

TABLE 2

Indices of electrophoretic variation and population differentiation (range) for red drum, other examined sciaenids (4 species), and Pacific shore fishes (10 species)

Group	$P_{(.01)}$	H	F_{ST}
Red Drum[a]	0.150	0.029	0.019
Sciaenids[a, b]	0.234 (0.171 - 0.368)	0.024 (0.009 - 0.043)	0.022 (0.014 - 0.032)
Shore Species[c]	0.379 (0.125 - 0.548)	0.031 (0.009 - 0.087)	0.045 (0.000 - 0.373)

[a] Ramsey and Wakeman (1987)
[b] Fujio and Kato (1979); Taniguchi (1980); Beckwitt (1983)
[c] Waples (1987)

Genic parameters for four polymorphic enzymes at various localities are listed in Table 3. The highest heterozygosity (0.035) was observed at Lake Borgne in Louisiana, and the lowest (0.022) was found at Mosquito Lagoon, Florida. Allelic frequencies revealed only weak patterns of geographic varia-

TABLE 3

Genic parameters for four polymorphic enzymes in red drum

Locality	n	Locus h				H(SE)
		s-Aat-A	Adh-A	Gpi-B	G-3pdh-A	
Texas						
Port Aransas	57	.228	.571	.070	297	.029(.017)
Louisiana						
Lake Charles	11	.182	.727	.000	.298	.030(.020)
Marsh Island	54	.208	.611	.093	.298	.030(.017)
Calliou Lake	38	.270	.474	.135	.324	.030(.016)
Terrebonne Parish	77	.186	.478	.039	.348	.026(.015)
Grand Isle	44	.182	.500	.159	.349	.030(.016)
Bay Gardene	56	.143	.521	.091	.378	.028(.016)
Lake Borgne	51	.157	.608	.176	.460	.035(.019)
Mississippi						
Biloxi Bay	34	.206	.471	.000	.500	.029(.018)
Florida						
Carrabelle	39	.205	.500	.026	.471	.030(.017)
Sarasota Bay	32	.219	.375	.031	.406	.026(.014)
Mosquito Lagoon	24	.042	.625	.000	.208	.022(.016)
St. Augustine	20	.150	.700	.000	.368	.030(.020)
h		.183	.551	.063	.362	
F_{is}		- .030	- .050	-.065	- .004	
F_{ST}		.014	.018	.030	.019	

tion, with 99% of the genetic diversity being within population samples, and only 0.2% within regions and 0.2% due to differences among regions. The range in values of the fixation index (F_{is}; deviation from Hardy-Weinberg frequencies) was small; the mean F_{is} (-0.03) indicated only slight deviations from panmixia and overall excess heterozygotes.

Adh-A allelic frequencies from various coastal locations indicate two major alleles along the Gulf of Mexico coast — Adh-A^{-60} and Adh-A^{-100} (Fig. 2). Rarer Adh alleles are scattered, showing no tendencies for geographic clumping. The only discernable geographic pattern is a significant change (contingency chi-square test) in the relative frequencies of the two major alleles in the

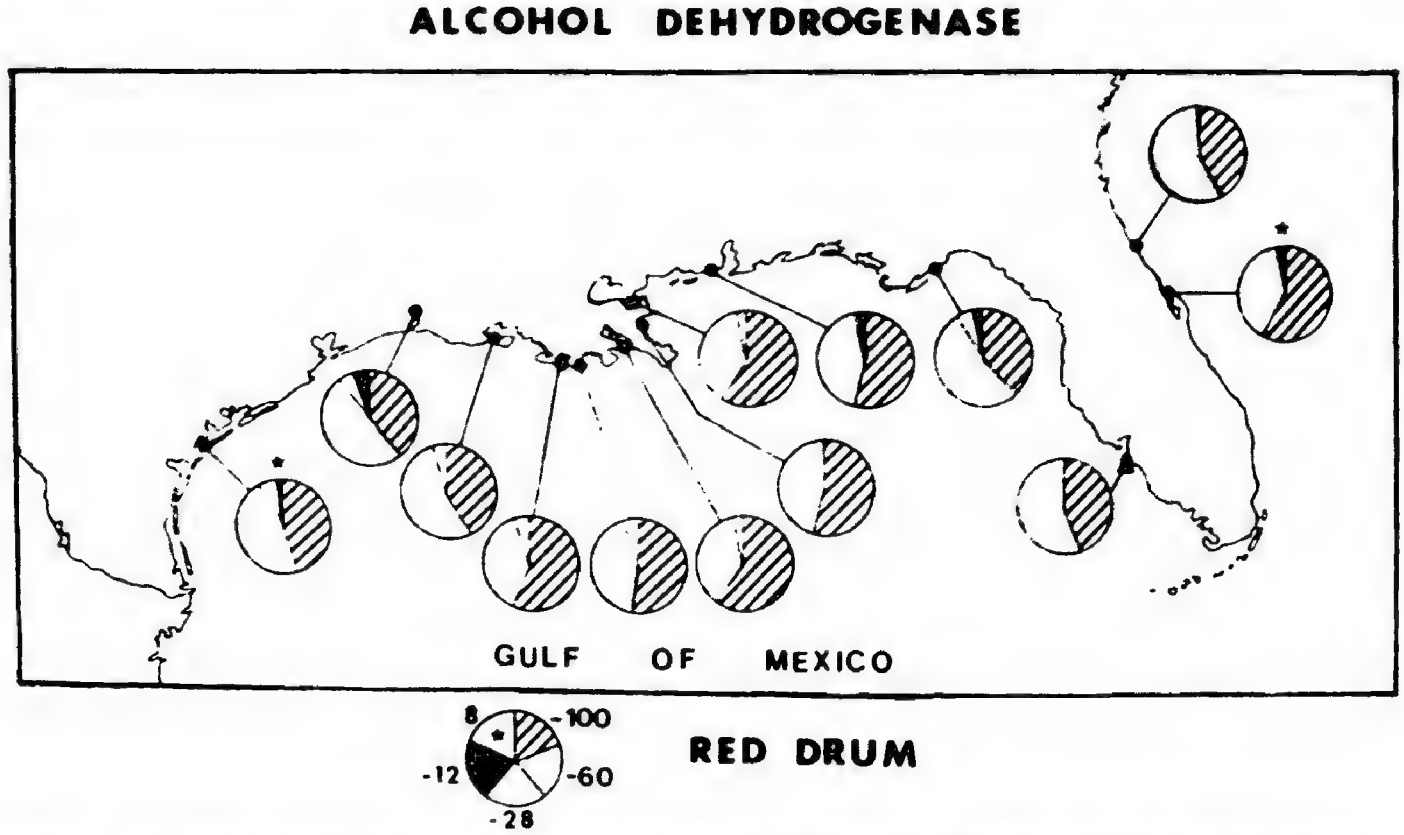

FIG. 2. Pie diagrams illustrating allelic frequencies for one polymorphic enzyme in red drum.

northern Gulf between the center of the Louisiana coast (Marsh Island) and Mobile, Alabama (Ramsey and Wakeman 1987). Throughout this area, Adh-A^{-100} occurred with greater relative frequencies than in other localities along the Gulf coast. If this pattern proves to be temporally stable, it could be due to differential selection on developmental stages in the low salinity marshes of this area, or it may indicate separation of offshore spawning schools into regional groups having different Adh allelic frequencies. Further studies are needed to ascertain the temporal stability of the Adh^{-100}/Adh^{-60} distribution patterns and the relative frequency of these alleles in offshore schools.

Occurrence of multiple tailspots (presumably genetically determined) in collected fish also indicated a mosaic pattern of geographic variation; percentages of fish with two or more ocelli ranged from 8% to 27% at various localities within Louisiana. The mean frequency of multiple tailspots was more than 6% lower in samples west of the Mississippi River, but the difference was not significant.

Condition factors ($100W/SL^3$) of collected fish were averaged according to season and location. Seasonal condition factors were lowest in spring (overall mean = 1.8 ± 0.02 SE) and highest in summer (overall mean = 1.99 ± 0.01 SE). However, the fish collected from the Port Aransas area of Texas during summer months had an average condition factor of only 1.82, indicating that red drum from this area were significantly less robust than those taken in summer collections from other Gulf states. Condition factors are presumably influenced primarily by environmental and/or physiological stresses. Reasons for the lower condition factors observed in the Texas population are unknown.

CONCLUSIONS

Because red drum populations are relatively large, the low genetic diversity observed along the Gulf coast from Florida to Teas is not unexpected, despite the fact that subadults of this species tend to remain in a single bay system during the first three or four years of their lives. Migration rates on the order of 1-5% per year among large populations are sufficient to maintain relative homogeneity. Moreover, mature individuals are known to migrate offshore where large schools of adult red drum are frequently observed. If breeding occurs in randomly migrating offshore schools, Gulf-wide panmixia would be expected.

In this study we have identified several rare alleles which could prove useful as markers or genetic "tags." These alleles include Adh-A^{-28}, Adh-A^{-12}, Adh-A^{8}, and 3 rare alleles for s-Aat-A as described by Ramsey and Wakeman (1987). After tissue biopsy and electrophoretic screening, the high fecundity of red drum could be exploited to produce large numbers of fingerlings with one of these alleles. If such fingerling were released into bay systems for stock enhancement purposes, their survival and dispersal could be monitored electrophoretically.

ACKNOWLEDGMENTS

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STUDIES ON THE BASIC STRUCTURE OF THE RED DRUM (*SCIAENOPS OCELLATUS*) GENOME

J.R. Gold, K.M. Kedzie, D.A. Bohlmeyer, J.D. Jenkin, W.J. Karel, N.Iida and S.M. Carr

Department of Wildlife and Fisheries Sciences
Texas A&M University
College Station, Texas 77843

ABSTRACT

Several basic parameters of the red drum (*Sciaenops ocellatus*) nuclear and mitochondrial genomes have been investigated as the initial phase of a long-term study of red drum genetics. (1) The red drum karyotype contains 2n = 48, differently-sized acrocentric chromosomes and a single pair of nucleolus organizer region (or NOR) bearing chromosomes. (2) The red drum genome size (DNA content) is estimated to be 1.65 ± 0.01 picograms of DNA per diploid nucleus. (3) The base composition of red drum nuclear DNA is 41.6 ± 0.1% GC (guanine-cytosine) base pairs; compositional heterogeneity and asymmetry values are 8.8 ± 0.2 and 2.3, respectively. The melting rate profile of red drum nuclear DNA indicates the presence of a highly repeated or satellite DNA family enriched in AT (adenine-thymine) base pairs. (4) Starch-gel electrophoresis of red drum tissue proteins has revealed five polymorphic or variable systems among the 27 putative gene loci now well resolved. (5) Highly purified red drum mitochondrial (mt) DNAs have been examined for digestion patterns using type-II restriction endonucleases. The size of the red drum mtDNA molecule is approximately 16.8 ± 0.2 kilobase pairs. An approximately 9.2 kilobase pair fragment of the red drum mtDNA has been cloned into a plasmid vector. The red drum mtDNA has been partially mapped.

INTRODUCTION

The red drum or redfish (*Sciaenops ocellatus*) is an important sport and commercial fish along the U.S. Gulf and Atlantic coasts. The apparent decline in the red drum fishery since the peak years between 1973-1978 (Matlock 1984) has caused widespread concern and accentuated the need for research on both wild and domesticated red drum populations, especially in the area of genetics. In this report, several basic parameters of the structure of the red drum nuclear and mitochondrial genomes are described as part of the initial phase of a long-term study of red drum genetics.

MATERIALS AND METHODS

Red drum samples for the research were obtained from four sources:

(i) Drs. Ed Robinson and Bill Neill of Texas A&M University and their projects on red drum nutrition and physiology;

(ii) Dr. Duncan MacKenzie of Texas A&M University and his project on red drum endocrinology;
(iii) Dr. Tom Linton of Texas A&M University and his Marine Advisory Service project with Dr. Jack Castle and Mr. Les Appelt of Silver Creek Farm near Palacios, Texas; and
(iv) a salt marsh near Grand Isle, Louisiana.

The samples from Drs. Robinson, Neill, and MacKenzie were obtained fresh. The samples from Silver Creek Farm were obtained by gill netting; those from Grand Isle were obtained by angling. Appropriate tissues were removed from both Silver Creek Farm and Grand Isle fish in the field and frozen in liquid nitrogen. All fish from Texas (i-iii) came from the Perry R. Bass Marine Fisheries Research Station near Palacios, Texas, and represent spawn from the John Wilson Marine Fish Hatchery at Flour Bluff, Texas. Both facilities are under the direction of the Texas Parks and Wildlife Department. The fish from Louisiana presumably represent spawn from a wild population.

Chromosome preparation and staining followed the methods of Gold (1984) and Amemiya and Gold (1987) employing live fish and gills as the tissue source. Photomicrography and computer-assisted measurement of chromosomes followed Gold and Amemiya (1986). Genome size (DNA content) measurements employed flow cytometric analysis of erythrocytes using live fish and following methods outlined in Bickham, Tucker and Legler (1985). Chicken erythrocytes were used as a DNA quantity standard. Isolation, purification, and thermal denaturation of nuclear DNA followed Karel and Gold (1987). Starch-gel electrophoresis of proteins followed procedures described in Morizot and Siciliano (1984) and employed muscle, liver, eye, and brain tissues which had been frozen in liquid nitrogen. Purified red drum mitochondrial (mt) DNAs were obtained from fresh liver and kidney tissue, digested with type-II restriction endonucleases (both singly and in pair-wise combinations), radioactively end-labeled, separated electrophoretically in agarose or polyacrylamide gels, baked onto filter papers, and autoradiographed. A few restriction sites were mapped by comparison of the single and double digestion patterns of specific enzymes. The methods used followed Carr and Griffith (1987) and Carr, Brothers and Wilson (1986). An approximately 9,200 base pair fragment of red drum mtDNA was inserted into a pTZ plasmid vector and cloned in *E. coli* strain DH5 after Maniatis, Fritsch and Sambrook (1982) and Hanahan (1984). Several restriction sites within the insert were mapped by removal of the insert from the vector, digestion with restriction endonucleases as before, separation of fragments using agarose electrophoresis, and UV-visualization of banding patterns after staining with ethidium bromide.

RESULTS AND DISCUSSION

The red drum karyotype consists of $2n = 48$, differently-sized acrocentric chromosomes and a single pair of nucleolus organizer region (or NOR) bearing chromosomes (Fig. 1). The NORs are the chromosomal sites of the ribosomal RNA genes, and in some fishes vary considerably both within and between species (Gold and Amemiya 1986). No variation in chromosome number or type, or in the number of chromosomal NORs, was observed among 20 individuals examined. The $2n = 48$ acrocentric karyotype and single pair of NOR-bearing chromosomes is fairly typical of a perciform fish (LeGrande 1987; Amemiya and Gold unpublished). The red drum chromosomal NOR, however, is situated sub-centromerically on the long arm of its chromosome (Fig. 1c). In most fishes, the (typically) single pair of NORs are situated terminally on the short arm of a submeta- or acrocentric chromosome (Gold and Amemiya 1986; and others).

The genome sizes (DNA contents) of eight individuals ranged from 1.57 - 1.69 picograms per diploid nucleus ($\bar{x} = 1.65 \pm 0.01$). The differences

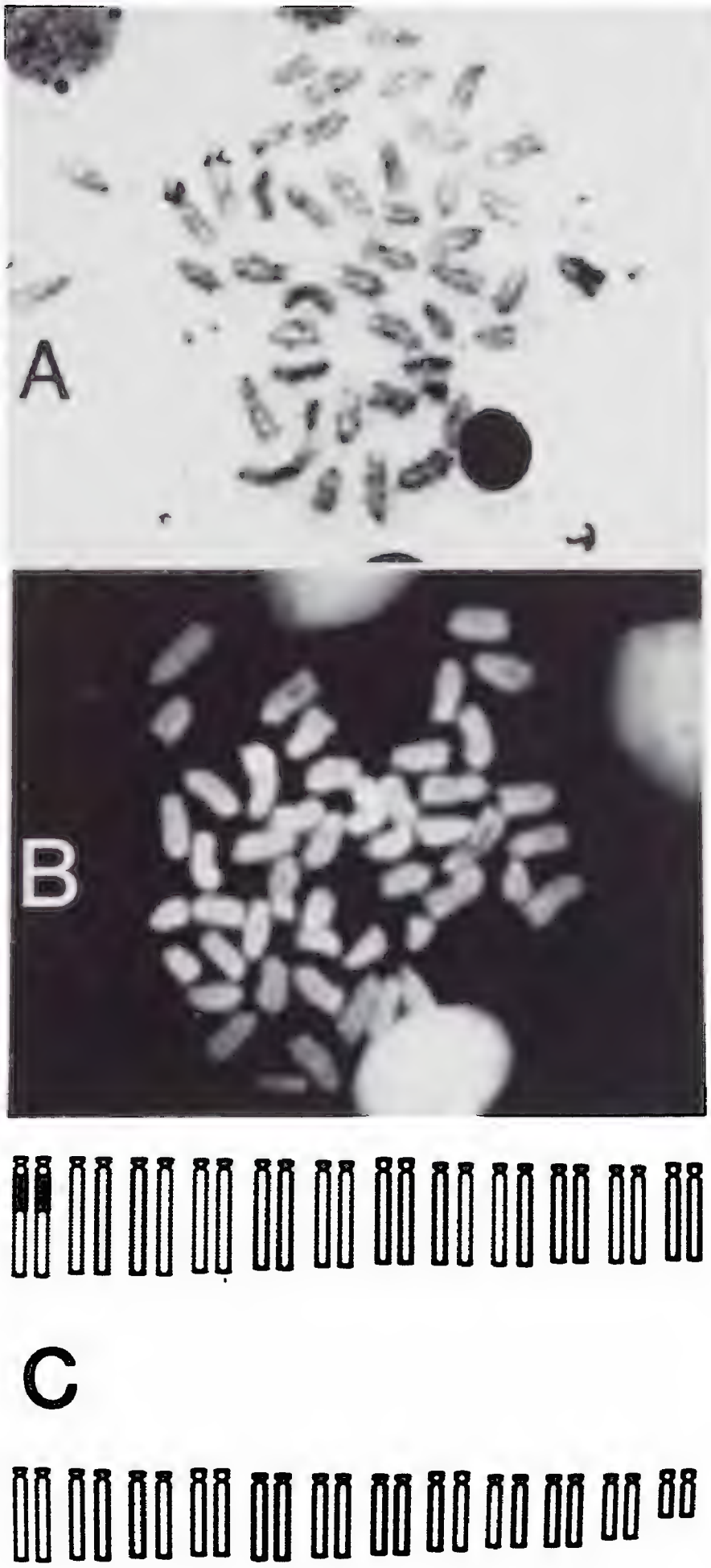

FIG. 1. (A) Silver- and (B) chromomycin A3-stained metaphase chromosomes from *Sciaenops ocellatus* showing the single pair of chromosomal nucleolus organizer regions or NORs. (C) Computer-assisted idiogram (from digitized measurements) of the *S. ocellatus* diploid karyotype; chromosomal NOR positions are indicated by darkened areas.

between individuals ranged from 0-6% and averaged around 3%. This level of genome size variation between individuals within populations is fairly typical of teleost fish (Gold and Amemiya 1987). The genome size of the red drum falls well within the range observed for other perciform fish (Hinegardner and Rosen 1972).

The base composition of red drum nuclear DNA is 41.6 ± 0.1% GC (guanine-cytosine) base pairs as estimated by thermal denaturation; the compositional heterogeneity and asymmetry values of red drum DNA are 8.8 ± 0.2 and 2.3, respectively. These values (including base composition) are well within the range found in other teleost fishes (Hudson, Cuny, Cortadas, Haschemeyer and Bernardi 1980; Karel and Gold 1987). The melting rate profile of red drum nuclear DNA is shown in Figure 2. The prominent, minor

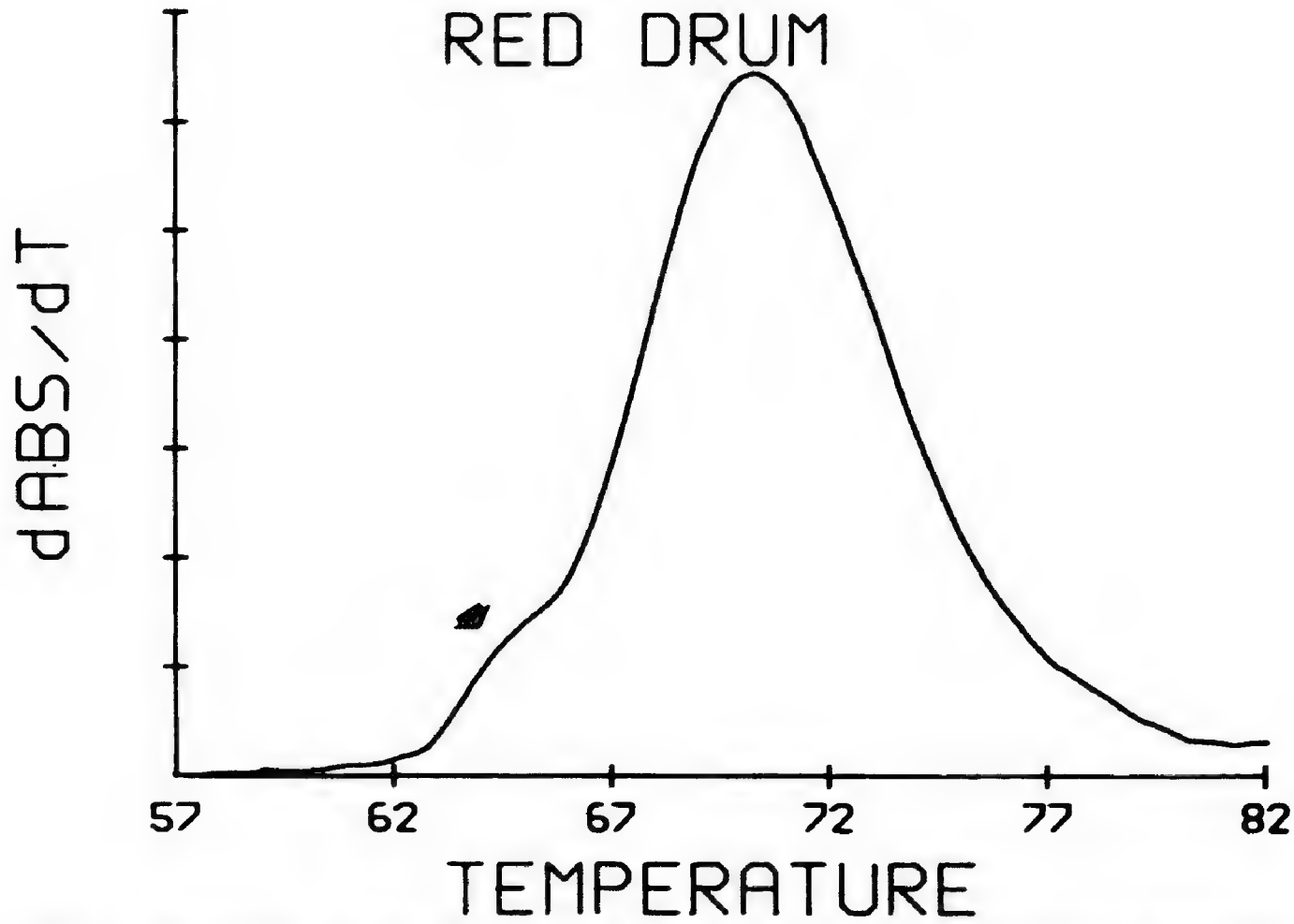

FIG. 2. Differential melting rate profile of red drum nuclear DNA. Abscissa: denaturation temperature; ordinate: increase in absorbance for each step increase (0.2°C) in temperature. The minor peak, possibly representing a family of repeated DNA sequences, is indicated by an arrow.

peak indicates the presence in the red drum genome of a highly repeated or satellite DNA family enriched in AT (adenine-thymine) base pairs. The estimated base composition of this putative satellite DNA family is 26.4 - 26.6% GC.

Systems for 27 presumptive red drum protein-coding gene loci are now well resolved. The enzymes, loci, tissues employed, and buffers used are shown in Table 1. Systems for another 15-20 loci show varying degrees of staining

TABLE 1

Protein Coding Loci Resolved

Enzyme	Enzyme commission number	Tissue source*	Buffer system**
Aconitase (ACON)	4.2.1.3	L	1
Adenosine deaminase (ADA)	3.5.4.4	M	2
Adenylate kinase (AK)	2.7.4.3	M	2
Alcohol dehydrogenase (ADH)	1.1.1.1	L	1
Creatine kinase (CK)	2.7.3.2	M	2
Enolase (ENO)	4.2.1.11	B,E	1
Esterase (ES-1)	3.1.1.1	M	1
Esterase (ES-2)	3.1.1.1	M	1
Esterase (ES-3)	3.1.1.1	M	1
Fumarase (FUM)	4.2.1.2	M	3
α Glycerophosphate dehydrogenase (αGPD)	1.1.1.8	M	2
Glyoxylase (GLO)	4.4.1.5	M	1
Isocitrate dehydrogenase (IDH)	1.1.1.42	L	1
Lactate dehydrogenase (LDH-1)	1.1.1.27	B,E	2
Lactate dehydrogenase (LDH-2)	1.1.1.27	B,E	2
Lactate dehydrogenase (LDH-3)	1.1.1.27	B,E	2
Malic enzyme (ME)	1.1.1.40	M	1
Mannose phosphate isomerase (MPI)	5.3.1.8	M	2
Peptidase (PEP B)	3.4.11	M	1
Peptidase (PEP D)	3.4.11	M	2
Peptidase (PEP S)	3.4.11	M	1
Phosphoglucomutase (PGM)	2.7.5.1	M	2
Phosphoglycerate kinase (PGK)	2.7.2.3	M	2
Pyruvate kinase (PK)	2.7.1.40	B,E	1
Superoxide dismutase (SOD)	1.15.1.1	B,E	2
Triosephosphate isomerase (TPI)	5.3.1.1	B,E	1
Uridine monophosphate (UMPK)	2.7.4	B,E	2

* L = liver; M = muscle; B,E = brain, eye.
** 1 = Tris-versene-borate, pH 8.0; 2 = Tris-citrate, pH 7.0; 3 = Ridgeway.

activity, and require further work. Of the resolved systems, five loci have been found to be polymorphic (Table 2); additional polymorphisms are apparently present among the unresolved systems. Allele frequencies at the polymorphic loci have not been calculated since the primary focus has been on locus resolution.

Highly purified mitochondrial (mt) DNAs were isolated from several individuals and examined for digestion patterns using 25-30 type-II restriction endonucleases. Fifteen enzymes cut the red drum mtDNA and 35-40 restriction sites have been identified by end-labeling. The latter method was used to map a few sites, including the two Sst II sites (Fig. 3) which apparently

TABLE 2

Polymorphic Loci Resolved

Locus	# Alleles
Aconitase	2*
Adenosine deaminase	7
Alcohol dehydrogenase	3
Esterase - 1	2
Isocitrate dehydrogenase	2*

* Rare (< 0.05) allele.

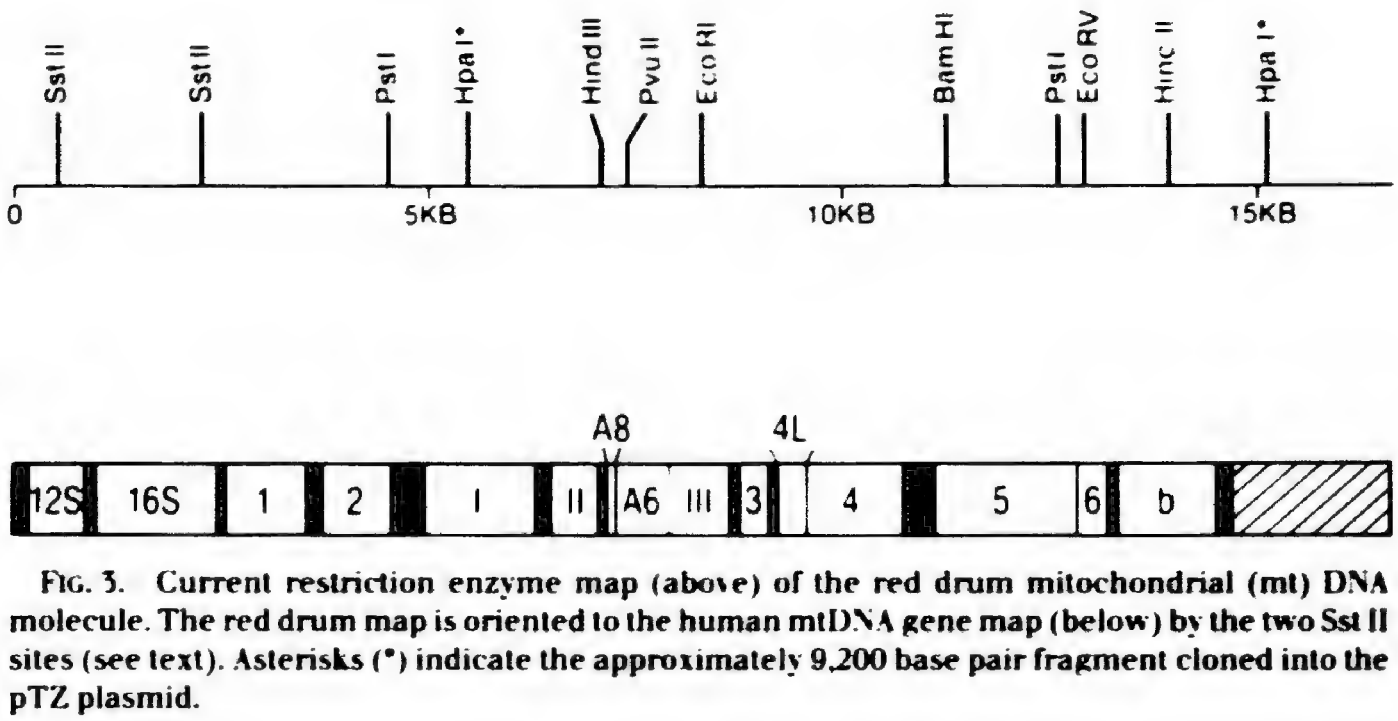

FIG. 3. Current restriction enzyme map (above) of the red drum mitochondrial (mt) DNA molecule. The red drum map is oriented to the human mtDNA gene map (below) by the two Sst II sites (see text). Asterisks (*) indicate the approximately 9,200 base pair fragment cloned into the pTZ plasmid.

are conserved throughout vertebrates and can be used to orient most vertebrate mtDNA maps to the mtDNA gene map in humans (Carr *et al.* 1986). Based on these experiments, the red drum mtDNA was estimated to be 16,800 ± 200 base pairs in length. Additional sites shown in Figure 3 were generated from mapping experiments using the approximately 9,200 base pair red drum mtDNA insert in the pTZ plasmid. No polymorphisms in size or restriction sites have yet been identified.

Data on genetic variability in red drum are few. Ramsey and Wakeman (1983) examined red drum from Louisiana and Texas for allelic variation at 41 presumptive gene loci; only three loci (including glucose phosphate isomerase or GPI) were demonstrably polymorphic. Attempts to resolve red drum GPI in our laboratory have been only partially successful. Satellite or artifactual bands often appear in GPI gels, regardless of the buffer employed, and no simple genetic models can yet explain the patterns of GPI variation observed. Wilder and Fisher (1986) surveyed the protein products of 30-40 loci from an unspecified number of red drum from Texas. They noted that

genetic variability was low, but did not give details as to the loci examined. The preliminary inference from the above is that red drum may not possess high levels of variability in nuclear protein-coding genes and hence "...illustrate the case where significant heterogeneity accompanies low population divergence" (Ramsey and Wakeman 1983). Whether or not protein electrophoresis will prove useful in discriminating red drum populations remains to be thoroughly tested. Mitochondrial DNA analysis, alternatively, holds great promise (Avise and Lansman 1983) for discriminating discrete red drum populations (should they exist), and current work in our laboratory is focused in that direction.

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CONTROLLED YEAR-ROUND SPAWNING OF RED DRUM *SCIAENOPS OCELLATUS* IN CAPTIVITY

C.R. Arnold

The University of Texas at Austin
Marine Science Institute
Port Aransas, Texas 78373

INTRODUCTION

The red drum *Sciaenops ocellatus* of the family Sciaenidae is a fish species occurring along the Gulf of Mexico and southern Atlantic coast. This euryhaline species is very important as a commercial and sport fish throughout its range. Natural spawning of the red drum occurs from late summer to early fall. Red drum spawn near the mouth of passes and the fertilized eggs, which are pelagic, flow into the estuaries on incoming tides. Texas Gulf coast temperatures during this time period range from 24 to 28°C.

One of the major problems which must be solved in the culture of finfish is the supply of fertilized eggs. In many instances the only source is stripping the eggs and milt from fish captured during their normal spawning time. In some instances gonadal hormones may be injected to cause ovulation and the fish may then spawn naturally or may be stripped. In either case this causes the loss of many immature oocytes and damage or death to the brood fish which could be of major significance if the brood fish are difficult to obtain. Each of these problems can be eliminated by maintaining healthy broodstock under conditions which produce a continuous supply of high quality eggs.

It has been demonstrated that temperature and photoperiod are two of the most important spawning stimuli in most fish species (Lasker 1974; Arnold, Lasswell, Bailey, Williams and Fable 1977; Arnold, Wakeman, Williams and Treece 1978; Roberts 1978 and Hussain, Akatsu and El-Zahr 1978). Temperature and photoperiod manipulation techniques have proved to be important tools used to induce maturation and spawning of finfish in the laboratory. After the temperature and photoperiod conditions for spawning a species have been determined, spawning may be delayed or prolonged by shifting the water temperature up or down from the optimum. Thus, temperature manipulation can be used to produce fertilized eggs on demand.

In this paper the methods used at the UTMSI Laboratory for induction of spawning and production of red drum eggs are presented. The value of temperature/photoperiod manipulations to marine finfish culture is demonstrated.

METHODS AND MATERIALS

Two male and two female wild caught red drum (10-15 kg) were placed in large spawning tanks (30,000 liters) equipped with biological filtration as described by Arnold, Bailey, Williams,

Johnson and Lasswell (1976). Photoperiod was controlled by using timers, and water temperature in the tanks was varied by manipulating ambient air temperature.

Temperature and photoperiod were set to correspond to the season in which fish were captured, and once acclimated to the captive environment, were cycled to reach the natural spawning season using 45 days for each season. Once the temperature and photoperiod reached the season in which they spawn naturally, these variables were held constant until spawning began (approximately 45 days). The photoperiod was then held constant and the temperature manipulated 6-8°C to retard or increase spawning frequency.

During all experimental manipulations fish were fed a diet of fresh frozen penaeid shrimp and fish at a rate of approximately 2% body weight every two days.

The four broodfish were cycled to reach a photoperiod of 12L:12D and a temperature of 26°C. Sex was determined by noting the intensity of drumming during transfer. Generally females do not drum or do so only weakly; sex was checked by inserting a catheter into the oviduct of the suspected females. After spawning began, (August 1980) the photoperiod was held constant, while the temperature was manipulated to retard or increase spawning. Tanks were monitored each evening for prespawning behaviors (Arnold *et al.* 1976) and each morning for eggs.

RESULTS

The first 41 months of spawning, averaged at monthly intervals, is shown in Figure 1. Although the large number of spawns during the first four months appears unusual, we have often observed high spawning frequency at the beginning of spawning cycles. On three occasions a spawn occurred every day for six consecutive days. The greatest frequency occurred in the 3rd month with a maximum of 20 spawns in 30 days.

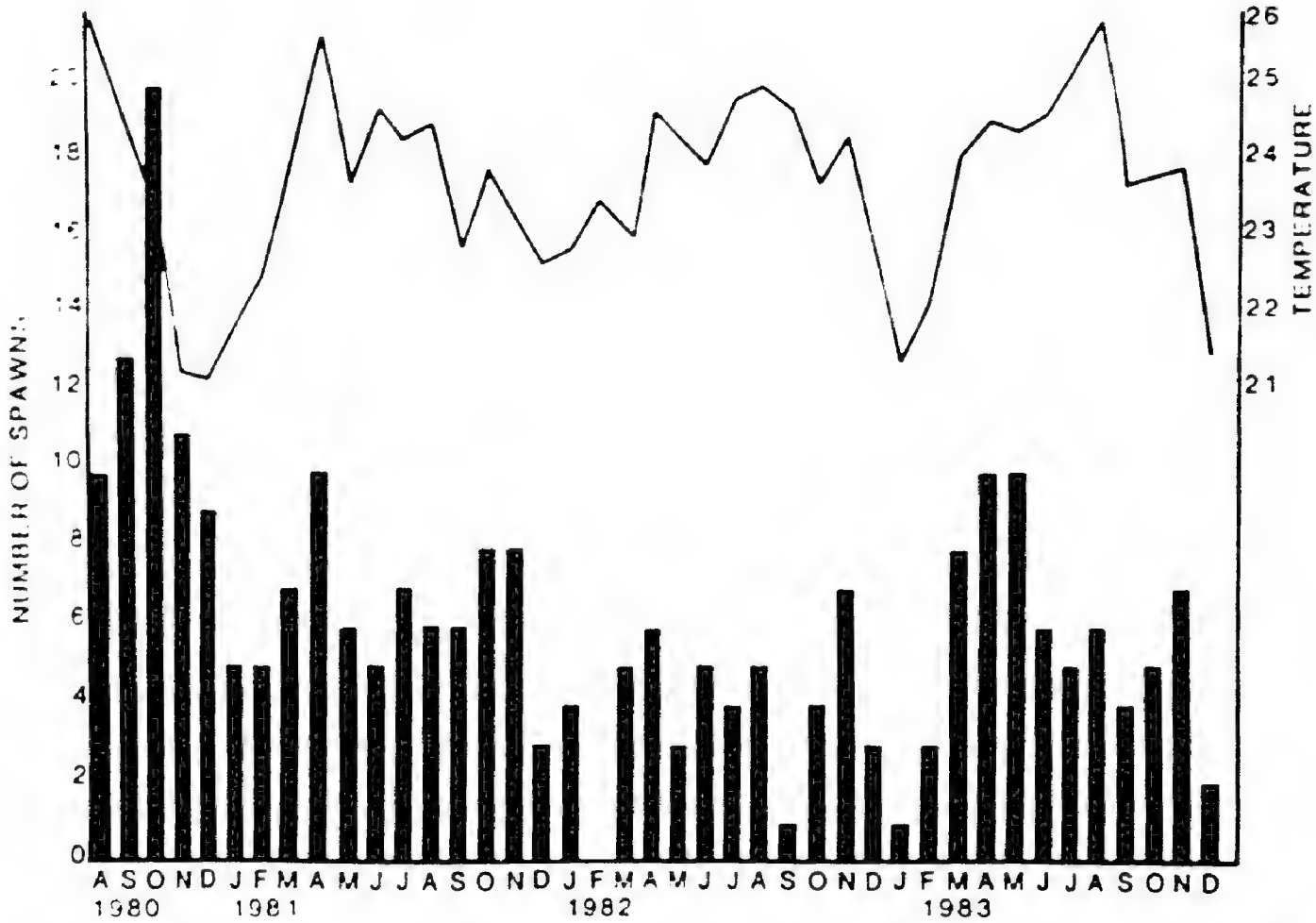

FIG. 1. Monthly spawning frequency (black bars) and average monthly temperature 1980-1983.

Frequent spawning early in the cycle can be altered by decreasing the temperature (Fig. 2). For example, six days of consecutive spawning was interrupted by a decrease in temperature of 2.8°C. Five days later as the temperature was increased from 21°C spawning resumed. This occurred during the 3rd and 4th month after the onset of spawning.

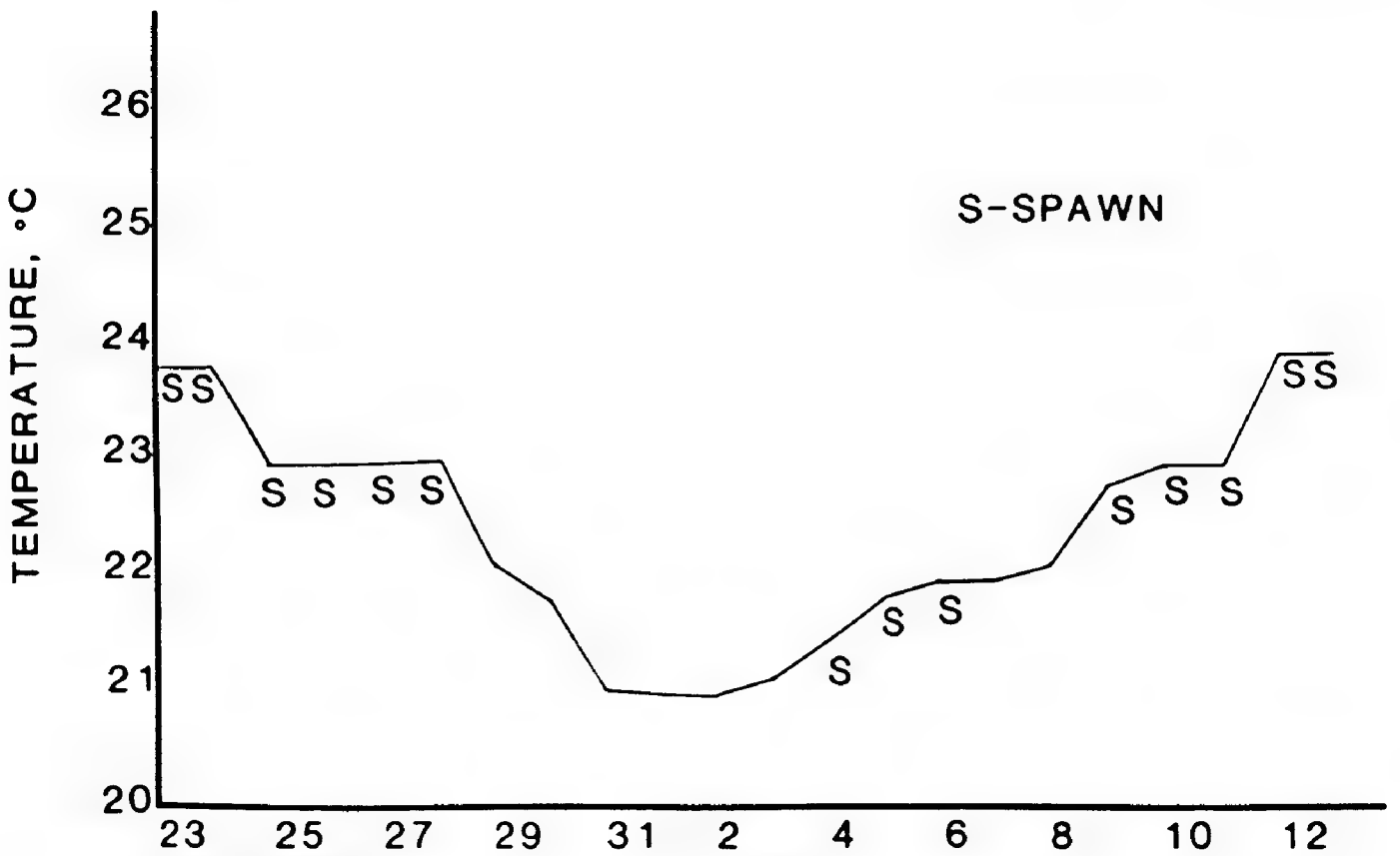

FIG. 2. Effect of lowering and increasing temperature on spawning frequency.

Drastically lowering the temperature caused spawning to cease. In December 1980 (5th month after initial spawning) there were nine spawns in the first 21 days. The last spawn was at 22.6°C on December 21 followed by rapid decrease in temperature to 17°C for the next six days (Fig. 3). This drastic lowering of the temperature was the result of outside low temperatures. The temperature remained below 20°C for 15 days before it began a rapid increase. Spawning resumed after the temperature was raised to 23°C, but spawning was interrupted for 25 days. This interruption apparently had no long term effect since spawning returned to an average of one spawn every five days.

In the temperature range of 20°C to 23°C we observed reductions in spawning frequency. Only 8.8% of the total spawns occurred at temperatures below 23°C, although these temperatures occurred 21% of the total time. In general no spawning occurred below 20°C. This agrees with our larval fish studies which showed little or no survival at 20°C (Holt, Godbout and Arnold 1981).

During the entire 41 month observation period, the only month in which spawning did not occur was February 1982 (Fig. 1); spawning ceased on January 26 and did not resume until March 7, a period of 41 days. It is difficult

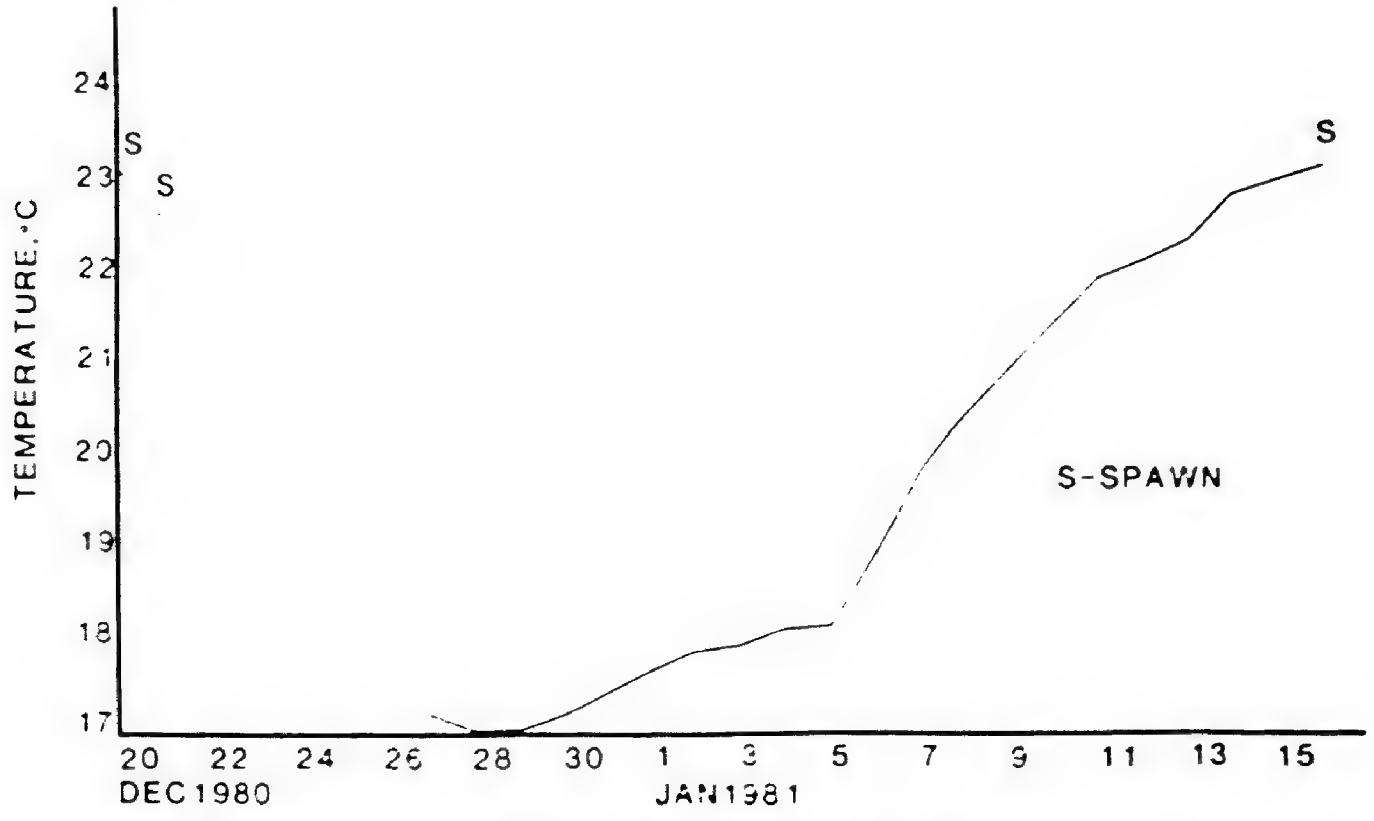

FIG. 3. Effect of drastic temperature reduction on spawning frequency.

to understand why this occurred since the temperature averaged 23.2°C and never went below 20.5°C during this period. This occurred during a 15 month period from December 1981 to February 1983 which had the lowest frequency of spawning; there were 51 spawns (3.4 spawns/month) which was below the overall average of 5.8 spawns/month. It is interesting to note the temperature during this time varied little (22.8 - 25°C), except for the last two months. Following these low temperatures the spawning frequency increased and stayed near the average until the end of 1983. The last spawn was on December 14, 1983 which ended the 41 month experiment. After this, temperature dropped to 17°C and stayed below 20°C most of January 1984. As a result no spawns occurred in January or February 1984.

Spawning did not occur again for over two months until March 1984 when they spawned two times. With an increase in temperature spawning reached a peak of 7 spawns per month (Fig. 4) two months later, May, 1984. A drop in the temperature slowed the spawning rate to 3/month then to zero for the next month. Spawning frequency was low for the next 5 months although the average temperature was slightly over 24°C. The temperature then dropped to a low of 21°C and spawning ceased for 5 months. Spawning began very slowly with a gradual increase in temperature reaching a total of 6 spawns per month after the temperature reached 26°C in July 1985. A slight drop in temperature resulted in a large drop in spawning frequency then increased to a frequency of 6 per month for 3 months. The temperature again dropped to a low of 21°C and spawning ceased. The fish were then removed for 3 months to install new spawning facilities. After returning the fish to the laboratory spawning resumed with a frequency of 7 spawns/month.

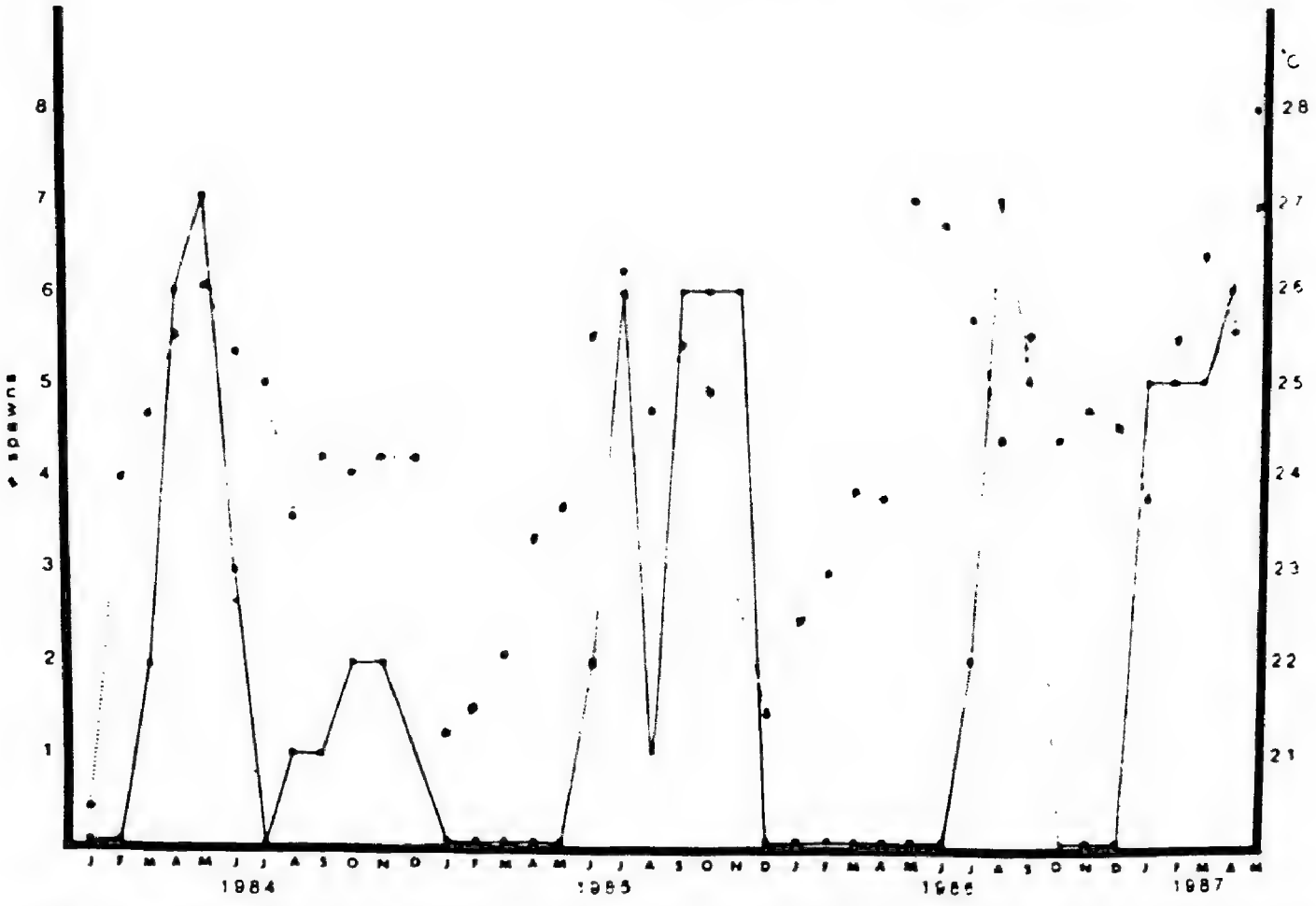

FIG. 4. Monthly spawning frequency (solid line) and average monthly temperature (dotted line) 1984-1987.

The first 16 months of this experiment produced 136 spawns, averaging 8.5 spawns/month or a spawn every 3.5 days. Each spawn produced a large number of eggs (50×10^3 to 2×10^6 with an average of 0.4×10^6).

In order to estimate the number of spawns and the number of eggs produced by one female red drum in a normal spawning season, the number of spawns were counted from the beginning of August 1980 through November 17, 1980. This is about the length of their normal spawning season. There were a total of 58 spawns during this time period by two females. This gives an estimate of 29 spawns/season/fish assuming that the two females never spawned on the same day. However, if both females spawned on the same day the number of spawns could be 58 spawns/fish/season. A total of 30,200,000 eggs were produced for an average of over 520 thousand eggs/spawn. A minimum estimate of egg production/season would be 15×10^6 per fish.

DISCUSSION

Throughout the seven year period, 1980-1987, the photoperiod was not changed from the 12L:12D cycle. These fish were not recycled because photoperiod conditions were being maintained for another tank of fish in the same room. Although these were not carefully planned studies, the resultant information is valuable in demonstrating the potential for long-term spawn-

ing which can be manipulated through temperature control. The two males and two females spawned 259 times from August 1980 to December 1983 and 101 times from March 1984 to June 1987 for a total of 360 spawns in 71 months, producing approximately 250 million fertilized eggs. This remarkable reproductive capacity shown by the red drum over the 7 year period may be unique among fishes and definitely projects red drum to the forefront of those species considered valuable for aquaculture. In summary this study shows that:

1. Red drum can be regulated to spawn indefinitely without going through a refractory period,
2. Red drum spawning frequency can be altered by temperature manipulations, and
3. The highest frequency of spawning occurs at temperatures above 23°C and does not occur below 20°C.

ACKNOWLEDGMENTS

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DIFFERING REPRODUCTIVE LIFE HISTORIES BETWEEN TEMPERATE AND SUBTROPICAL GROUPS OF *CYNOSCION NEBULOSUS*

Nancy Brown-Peterson[1] and Peter Thomas

The University of Texas at Austin
Marine Science Institute
Port Aransas, TX 78373

ABSTRACT

Differences in the reproductive biology of spotted seatrout, *Cynoscion nebulosus*, from temperate Chesapeake Bay, Virginia and subtropical Port Aransas, Texas were examined. Females became sexually mature at 232 mm SL in Texas *vs.* 267 mm SL in Virginia. All males collected in both areas were sexually mature. The spawning season extended from May to mid-August in Virginia and from April to the end of September in Texas and was closely related to the seasonal increase and decrease in water temperature. Gonadal development of males did not differ; > 80% of males were running ripe throughout the spawning season in both Virginia and Texas. In contrast, there were marked regional differences in the reproductive characteristics of female spotted seatrout. The mean gonadosomatic index of non-spawning females in the mature reproductive stage was consistently higher in Virginia fish throughout the reproductive season. Further, oocyte growth was more synchronous in fish in the mature reproductive stage from Virginia than in Texas fish. Atretic oocytes and postovulatory follicles were common in the ovaries of Texas fish, but rarely found in Virginia fish. In addition, spent or partially spent individuals were collected during the spawning season in Texas but not in Virginia. Together these results suggest that spotted seatrout are multiple spawners in Texas, whereas they may only spawn once in Virginia.

INTRODUCTION

Fishes with a broad geographical range often exhibit regional differences in their reproductive life histories (Fox 1978, Leggett and Carascadden 1978, Boehlert and Kappenman 1980). The spotted seatrout, *Cynoscion nebulosus*, ranges from temperate New York to subtropical Tampico, Mexico (Overstreet 1983). This warm water, estuarine species is abundant along the Gulf Coast of the United States and active breeding populations extend up the southeastern coast to Chesapeake Bay, Virginia (Brown 1981). However, it is not known whether spotted seatrout exhibits different reproductive characteristics throughout its geographical range in order to reproduce successfully under such a wide variety of environmental conditions.

Several differences can be expected in the reproductive biology of a species with a broad geographical range as the latitude increases. The spawning

[1] Current address: Florida Department of Natural Resources, Indian River Lagoon Aquatic Preserve, 4842 S. U.S. 1, Fort Pierce, FL 39482

season may be restricted to a few months of the year when environmental conditions are suitable for survival and growth of larvae. A shorter spawning season may result in greater synchrony of gonadal development and spawning within the population and more synchronous oocyte development and fewer spawns in an individual. Several aspects of the reproductive biology of spotted seatrout from the temperate waters of Chesapeake Bay, Virginia and the subtropical waters of Port Aransas, Texas were compared in the present study to determine whether these differences are exhibited by this species.

MATERIALS AND METHODS

Chesapeake Bay, Virginia (37° 15.2′ N, 76° 32.4′ W) is a temperate, drowned river valley estuarine system with a wide average annual variation in water temperature (0-30°C). Submerged aquatic vegetation is present but not abundant. Spotted seatrout are seasonal residents in Chesapeake Bay, arriving in May when gonadal recrudescence is beginning and leaving in October after the gonads have regressed. Specimens were obtained from Ware River or Hungars Creek twice monthly by haul seining or gill netting by commercial fishermen during May through October of 1979 and 1980.

The Aransas Bay system of south Texas (27° 57′ N, 97° 04′ W) is a subtropical, coastal plain estuarine system, with average annual water temperatures ranging from 14-33°C. There are extensive areas of shallow water with abundant submerged aquatic vegetation. Spotted seatrout are year-round residents in the shallow bays. Specimens were gill netted (same mesh size as used in Virginia) weekly or twice monthly at Hog Island or Lydia Ann Channel from April 1982 through November 1985.

Standard length (SL) to the nearest mm and total weight to the nearest 10 g were recorded for all specimens. Ovaries were removed and weighed to the nearest 0.1 g. The gonadosomatic index (GSI) was calculated for each fish following the method of Shepherd and Grimes (1984). A portion of the anterior of each gonad was preserved in Davidson's fixative for histological processing following standard techniques. Maturity stages were as described in Brown-Peterson, Thomas and Arnold (in press).

RESULTS AND DISCUSSION

Females reached sexual maturity at a smaller size in Texas than in Virginia; 95% of the females were sexually mature by 271 mm SL in Texas *vs.* 305 mm SL in Virginia. However, the largest female captured in Virginia was 615 mm SL, whereas the largest female in Texas was 533 mm SL. These sizes correspond to previously reported ages of 15 years for Virginia fish (Brown 1981) and nine years or less for Texas fish (Maciena, Hatta, Linton and Landry 1987). Thus, fish in Virginia may have a potentially longer reproductive life span than fish in Texas.

All males captured in both Texas and Virginia were sexually mature. However, the smallest male captured in Virginia was 221 mm SL, while the smallest male in Texas was 196 mm SL. Males achieve sexual maturity at a smaller size than females in both the temperate and the subtropical habitat, a common occurrence throughout the range of the species (Mercer 1984).

The spawning season, as determined by high GSI values in both males and females, is 2.5 months longer in Texas than Virginia (Fig. 1); spawning began

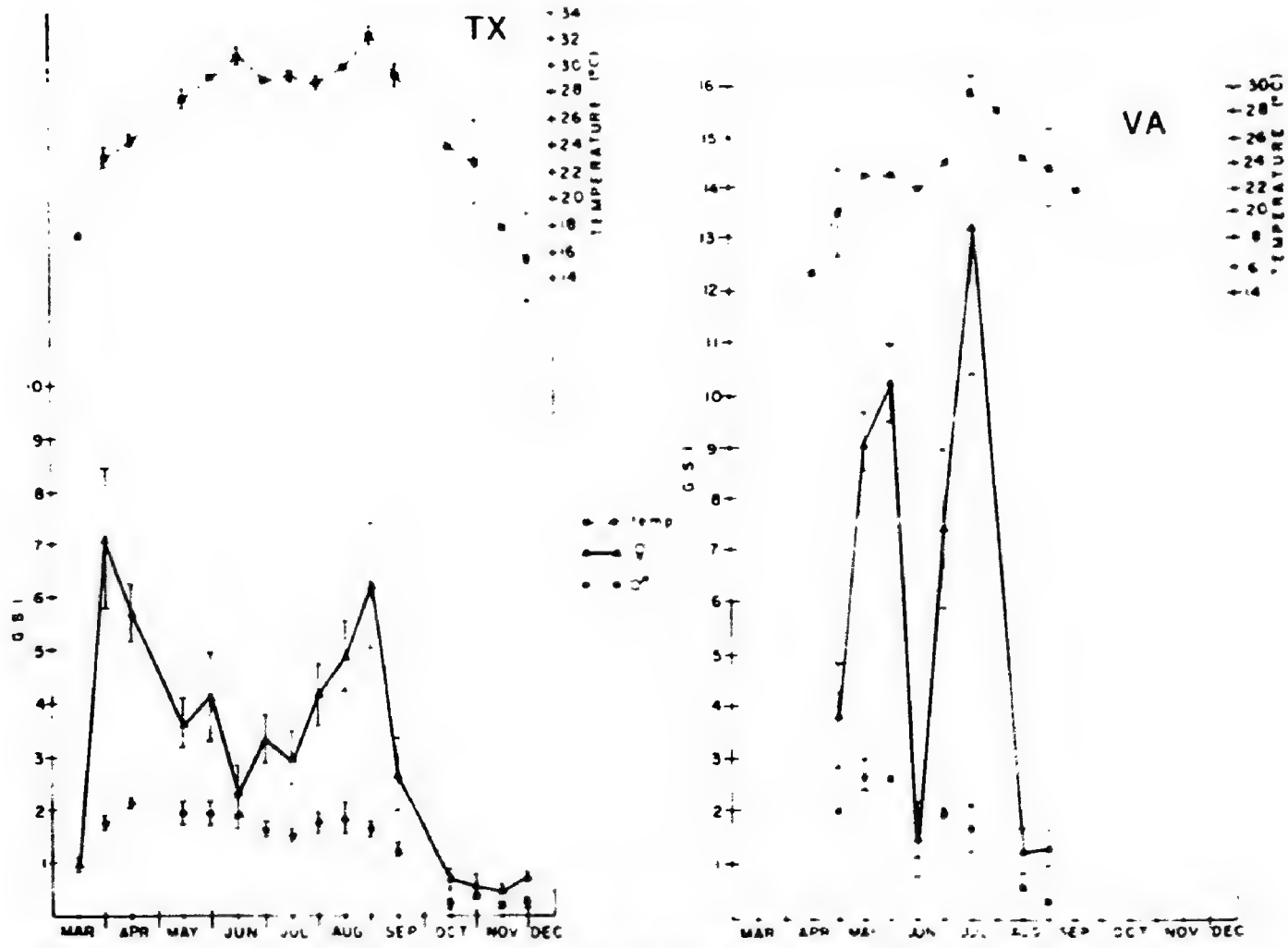

FIG. 1. Bi-monthly mean water temperature and mean gonadosomatic index (GSI) of male and female spotted seatrout collected in Port Aransas, Texas (TX) and Chesapeake Bay, Virginia (VA), including ± 1 standard error of the mean.

in April and ended by the first of October in Texas and began in May and ended in mid-August in Virginia. In Texas, gonadal recrudescence began in late January in males and in mid-February in females. In Virginia, however, fish did not arrive in Chesapeake Bay until May, at which time gonadal recrudescence had started in both sexes. Unfortunately, no data are available on Virginia fish prior to their arrival in Chesapeake Bay, so the time of the initiation of gonadal recrudescence is unknown. In both areas, there appear to be two peaks in female spawning activity. However, mean GSI values of non-spawning Virginia females in the mature reproductive stage were significantly greater (Student's t, $p < 0.001$, Fig. 2) than the GSI of similar size fish in Texas throughout the reproductive season. The mean size of the developing oocytes in the two groups of fish was similar, although histological observations showed more yolk globular oocytes present in the ovaries of Virginia fish (Table 1). Therefore, it appears that oocytes in a more advanced developmental stage are present in Virginia fish than in Texas fish at any time during the spawning season, which may indicate that Virginia females have a potentially higher fecundity per spawn than Texas females.

In Virginia as well as in Texas, females with ovaries in the mature reproductive stage were present in the bays one to two weeks prior to the

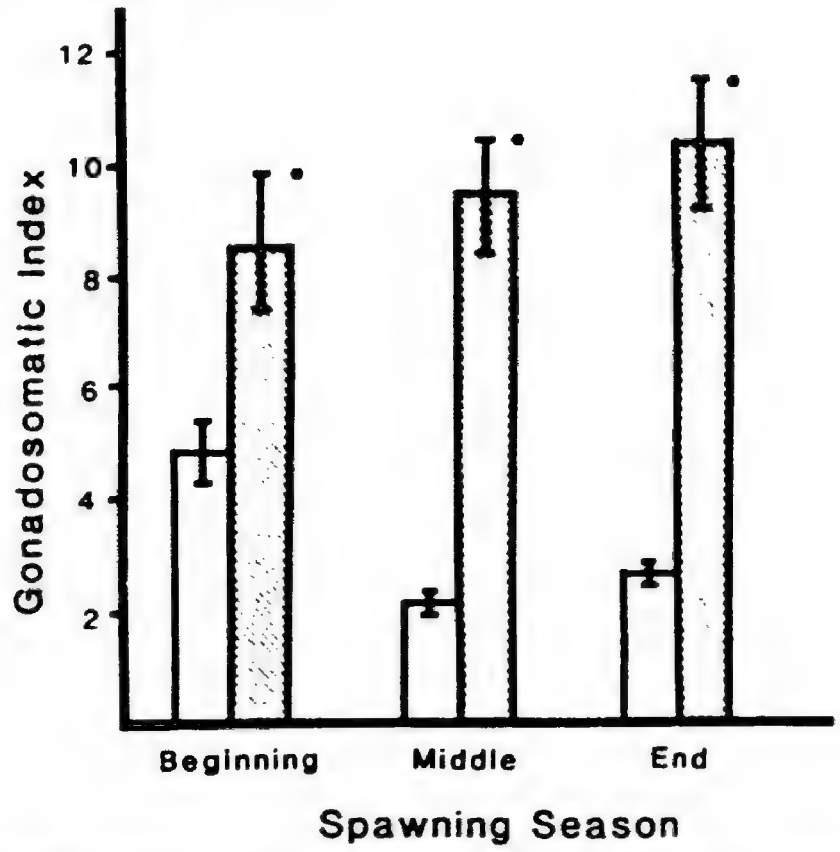

FIG. 2. Mean gonadosomatic index ($\bar{X} \pm 1$ SE, GSI) of non-spawning female spotted seatrout in the mature reproductive stage from Texas (hatched bars) and Virginia (open bars) at beginning, middle and end of spawning season. Asterisks (*) denote significant differences between the two populations. Number of Texas fish sampled at each time; 290, 58, 71, respectively. Number of Virginia fish sampled at each time; 22, 3, 16, respectively.

onset of actual spawning. However, the beginning of spawning was preceded by a rapid, 5-7°C increase in water temperature (Fig. 1). The temperature necessary to initiate spawning in the two areas was similar; 20°C in Virginia and 23°C in Texas. Spawning stopped in both areas following a decrease in water temperature to 24°C at the end of the summer. In both estuaries the spawning season began while daylength was increasing and ended as it was decreasing. However, these events occurred under different photoperiod conditions in the two estuaries.

Males in both Virginia and Texas maintained a condition of ripeness throughout the spawning season (Table 2). More than 80% of males in Virginia were running ripe from May through mid-August, while > 90% of males in Texas were running ripe from mid-March through the end of September. Thus, there does not appear to be any major differences in the reproductive biology of male spotted seatrout in Texas and Virginia.

In contrast to males, there appear to be differences in the reproductive characteristics of females in Texas and Virginia. A high percentage of Virginia females were mature or running ripe during the early and late portions of the spawning season, whereas most of them were in the early stages of ovarian development in the middle of the spawning season (Table 3). These data, together with the GSI results, suggest two peaks of spawning activity by Virginia fish, similar to those reported in Georgia, Florida and Louisiana

TABLE 1

Percentage of oocyte types in ovaries of fish from Virginia and Texas in the mature reproductive stage. Values ($\bar{x} \pm 1$ SE) compared with Student's t test (* $p < 0.05$; * $p < 0.01$; ** $p < 0.001$).

Time	Month	Area	N	% of Oocyte Types		
				Yolk Granule	Yolk Globule	Atretic
Early	May	VA	11	11.63 ± 0.69	32.00 ± 3.14	0.14 ± 0.10
	April	TX	58	13.60 ± 0.60	29.20 ± 1.00	5.50 ± 0.50**
Mid	June	VA	3	7.73 ± 1.67	39.87 ± 2.94	0.33 ± 0.33
	June	TX	17	7.30 ± 1.20	24.10 ± 2.80	5.37 ± 1.20
Late	July	VA	13	9.14 ± 1.48^{+}	38.97 ± 4.17*	0.36 ± 0.36
	Aug	TX	29	12.60 ± 1.30	27.30 ± 1.65	6.90 ± 1.00**

TABLE 2

Comparison of reproductive stages of male spotted seatrout from Chesapeake Bay, Virginia and Redfish Bay, Texas. Maturity assessed histologically. REG, Regressed; E DEV, Early Developing; DEV, Developing; MAT, Mature; RR Ripe and Running Ripe; SP, Spent.

Time	Month	Area	N	% in Each Development Stage					
				REG	E DEV	DEV	MAT	RR	SP
Early	May	VA	15	0	0	7	13	80	0
	April	TX	232	0	0	0	6	94	0
Mid	June	VA	7	0	0	0	14	86	0
	June	TX	36	0	0	0	3	97	0
Late	July-Aug	VA	28	0	0	0	7	68	25
	Aug-Sept	TX	131	0	0	0	0	92	8

(Mercer 1984). The majority of Texas females, however, were mature and running ripe throughout the spawning season, which suggests that spawning occurred throughout a six month period in Texas. Furthermore, Texas females in the mature reproductive stage had a consistently lower percentage of yolk globular oocytes and a higher percentage of yolk granule oocytes in their ovaries relative to Virginia fish, although this difference was only significant at the end of the spawning season (Table 1). Thus, oocyte development appears to be more asynchronous in Texas fish. Virginia fish had few atretic oocyte and post ovulatory follicles, whereas more than 5% of the oocytes were atretic in Texas fish (Table 1). These histological observations, together with the absence of spent and partially spent females

TABLE 3

Comparison of reproductive stages of female spotted seatrout from Chesapeake Bay, Virginia and Redfish Bay, Texas. Maturity assessed histologically. REG, Regressed; E DEV, Early Developing; DEV, Developing; MAT, Mature; RR, Ripe and Running Ripe; PSP, Partially Spent; SP, Spent.

				% In Each Development Stage						
Time	Month	Area	N	REG	E DEV	DEV	MAT	RR	PSP	SP
Early	May	VA	23	0	5	0	95	0	0	0
	April	TX	372	0	4	6	78	12	0	0
Mid	June	VA	13	0	61	15	24	0	0	0
	June	TX	104	0	0	1	56	19	24	0
Late	July-Aug	VA	31	10	13	13	51	13	0	0
	Aug-Sept	TX	137	4	0	0	52	32	8	4

in Virginia (Table 3), suggest that some individuals in Texas with mature ovaries had spawned previously and may spawn several times during the reproductive season, while Virginia individuals appear to spawn only once or twice. The August GSI peak in Virginia may indicate spawning of a late spawning group of females which had developing ovaries in June (Table 3).

The data presented show the expected differences in the reproductive biology between Texas and Virginia female spotted seatrout, although there appears to be no differences in male reproductive biology other than a shorter spawning season in Virginia. These findings are similar to observations on other species with extended reproductive ranges (Fox 1978, Conover and Kynard 1984, Hubbs 1985), and are probably a result of a shorter summer season in higher latitudes, with less time for growth, utilization of abundant food resources and optimal conditions for larvae and juveniles. Additionally, the differing reproductive characteristics observed may be related to a more energetically demanding environment in higher latitudes. Spotted seatrout must reserve energy to migrate from Chesapeake Bay each winter and may have less energy to allocate to reproduction, as suggested by Leggett and Carascadden (1978) for *Alosa sapidissima*. A similar explanation has been proposed for differences in the reproductive biology of *Cynoscion regalis* in the New York and South Atlantic Bights (Shepherd and Grimes 1984).

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MEASUREMENT OF OVARIAN DEVELOPMENT IN RED DRUM (*SCIAENOPS OCELLATUS*) FROM OFFSHORE STOCKS

Gary R. Fitzhugh[1], Theron G. Snider III[2], and Bruce A. Thompson

Coastal Fisheries Institute
Center for Wetland Resources
Louisiana State University
Baton Rouge, LA 70803-7503

ABSTRACT

Histological examination of ovaries from red drum (*Sciaenops ocellatus*) collected from offshore waters indicated multiple oocyte stages were present throughout the spawning season. Cortical alveolar and vitellogenic oocytes were common among females by July, indicating onset of reproductive development. Evidence of spawning was determined from presence of post-ovulatory follicles beginning in late August. Few females exhibited hydrated oocytes in collections possibly due to onset of hydration 3-5 hours prior to spawning. Heterogeneity of hydrated oocyte numbers by location within ovaries was noted and must be accounted for in future surveys involving batch fecundity determinations.

INTRODUCTION

Evaluation of gonad maturation will allow determination of reproductive status and the proportion of stock that is reproductively active. Previously, this has not been determined for offshore schools of red drum which reportedly may act as Gulf of Mexico "brood stock." Overstreet (1983) provided a detailed description of gonad development for juveniles and newly maturing adults but did not observe actively spawning females. Therefore it is important to examine fecundity as a function of size and age from adult fish residing predominately in offshore waters.

Recently, techniques refined to improve measurement of egg production has lead to wider recognition that many marine species are multiple spawners. Spawning frequency and clutch sizes produced must be determined for accurate fecundity models (Hunter, Lo and Leong 1985). Before fecundity methodology can be applied with precision for population estimation, survey of adult fish and assessment of ovarian development is necessary for the determination of spawning patterns and development of sampling criteria.

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[1] Current address: Department of Zoology, Box 7617, North Carolina State University, Raleigh, NC 27695

[2] Department of Veterinary Pathology, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803

MATERIALS AND METHODS

Monthly sampling of red drum from commercial catches was initiated in February 1986 for measurement of gonad development. Samples were collected as frequently as possible during the fall spawning season but were dependent on availability of adults via fishing tournaments and National Marine Fisheries Service research/tagging cruises.

Following measurement of body meristics and ovarian weight, the ovaries were fixed in 10% formalin. Batch fecundity or counts of hydrated oocytes comprising the leading "clutch" of eggs immediately prior to spawning were determined from tissue samples following Hunter *et al.* (1985).

To determine the accuracy of batch fecundity methodology (Hunter *et al.* 1985), two replicate ovarian tissue samples were taken from anterior, mid and posterior regions of left and right lobes from nine females exhibiting gross visual signs of hydration. Counts were expressed as eggs per gram of preserved ovarian tissue. Comparisons among individual females, right *vs.* left ovarian lobes within individuals, and locations within lobes among individuals were made using a nested Analysis of Variance model, SAS GLM procedure (SAS 1985) (Table 1).

TABLE 1

Effect of location of tissue samples for hydrated egg counts per unit of weight (g). Locations are anterior (i) mid (ii) and posterior (iii) of ovarian lobes. Analysis of variance indicates significance of location within a lobe for number of hydrated eggs per gram of tissue.

Positions of sample in ovary	Mean Number and Standard Deviation of Eggs/Gram of Ovary Tissue								
	Right Lobe			Left Lobe			Both Lobes		
	X	s	n	X	s	n	X	s	n
i	2,076.6	1,113.5	18	1,813.1	1,082.2	18	1,944.8	1,090.4	36
ii	1,901.9	889.1	18	1,639.2	1,235.7	18	1,770.5	1,069.3	36
iii	1,872.9	696.3	18	1,414.0	947.1	18	1,643.5	851.6	36
Total	1,950.5	902.8	54	1,622.1	1,086.5	54			

Nested ANOVA of eggs/gram of ovary tissue.

Source of Variation	df	MS	F	PR > F
Individual female	8	9,474,339.5	4.28	0.02
Right vs.left lobe (within individual)	9	2,214,407.2	8.12	0.0001
Location (within lobe by individual)	36	272,543.9	4.70	0.0001
Sampling error	54	57,988.7		
Total	107	12,019,279.3		

A tissue sample was removed from the mid region of the preserved right ovarian lobe of each female, embedded in paraffin, sectioned, stained with Gill hematoxylin and counter-stained with eosin for histological examination. Assessment of oocyte stages were made following Wallace and Selman (1981) and deVlaming (1983).

A stratified random sampling scheme was employed for stereological analysis where a random starting point was located on a section and all identifiable oocyte stages within a field of view were counted before moving to a new field using manual stage drive. Field movement was

inward along the ovigerous lamellae from the tunica albuginea toward the center of the ovary with realignment along a vertical axis (Weibel 1979). A minimum of 200 oocytes of the four developmental stages were counted, requiring 2 to 4 passes through the section and the numbers expressed as a percentage of the total (Htun-Han 1978, Holdway and Beamish 1985). Only cells containing greater than 50% of their area within a field of view were counted. The Bioquant IV image analysis system software, IBM PC and Houston Instrument digitizing pad (Hipad model DT-11) was used in conjunction with an Olympus microscope (with video attachment) to facilitate cell counts.

RESULTS AND DISCUSSION

Wallace and Selman (1981) present the various attributes of ovarian types relative to oocyte recruitment and spawning strategies. Oocyte recruitment for an upcoming reproductive season can occur at the end of a gonadotropin-independent stage (primary growth, PG) characterized by a year-round population of small basophilic-staining oocytes (Wallace and Selman 1981). Oocyte recruitment and seasonal gonadogenesis in red drum began as groups of primary growth (PG) oocytes, then passed into a cortical alveolar stage (CA) characterized by yolk vesicle formation. Cortical alveolar oocytes matured into vitellogenic (V) stages where uptake of yolk protein accounted for further oocyte growth (Wallace and Selman 1981). Only three of 33 females examined April through June, exhibited CA stage oocytes. Cortical alveolar and vitellogenic (V) stage oocytes were not common among individuals until July, exhibiting a mean of 1.8 and 1.4 percent respectively, of total eggs present (Fig. 1). Gonadotropin dependent cortical alveolar and vitellogenic stages

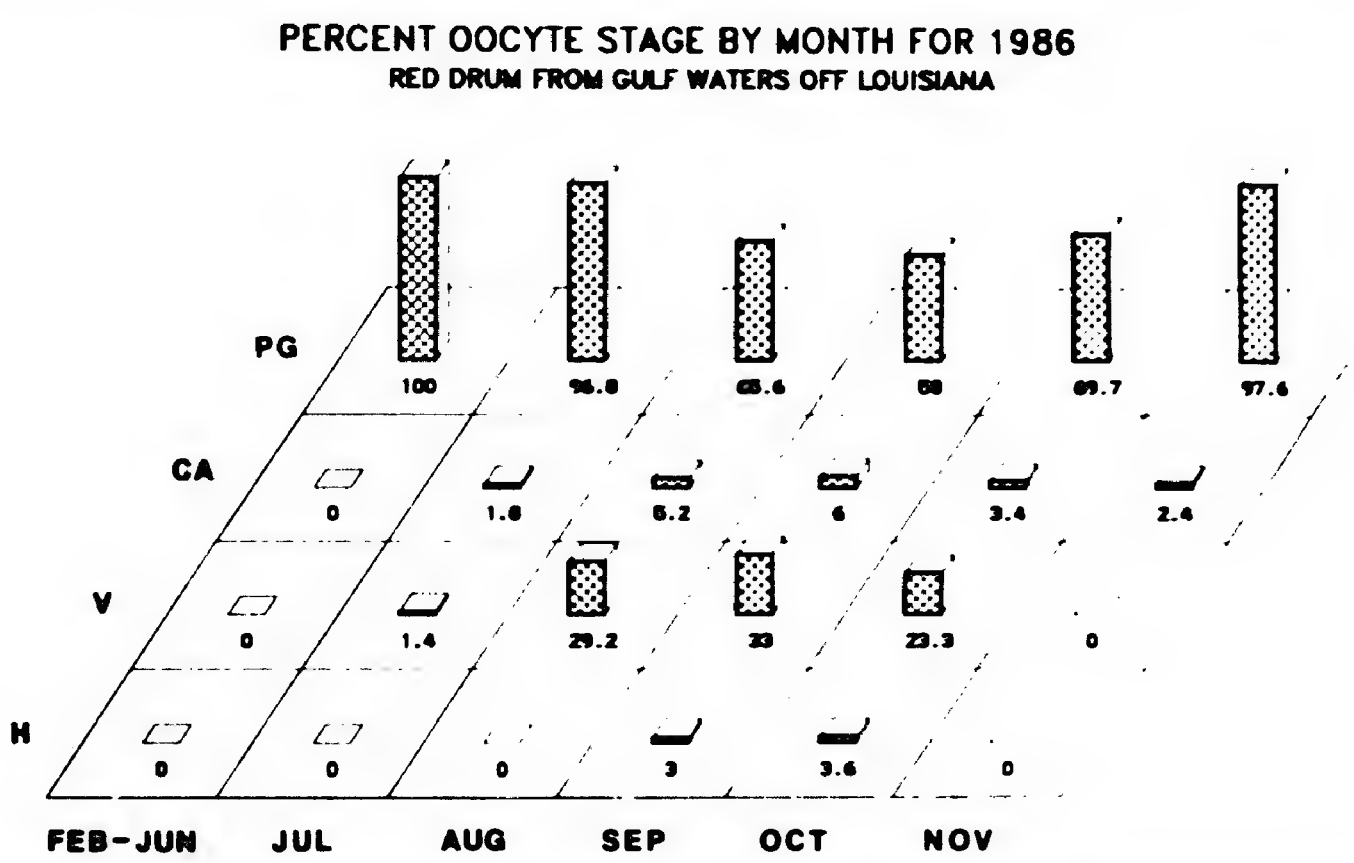

FIG. 1. Block chart of percent oocyte stage by month based on point counts of approximately 200 oocytes per female (n = 50, February-June; 9, July; 64, August; 39, September; 19, October; 2, November). Stages include primary growth (PG), cortical alveolar (CA), vitellogenic (V) and hydrated (H).

increased in frequency in August, September and October coinciding with the reported spawning season (Fig. 1). Recruitment from the gonadotropin-independent population of oocytes (primary growth) occurred throughout the spawning period as cortical alveolar stage oocytes were present at 2-6% of the total egg population July through October (Fig. 1). This pattern of recruitment of groups of large oocytes from a population of small sized oocytes distinguishes group-synchronous ovaries (Wallace and Selman 1981).

First evidence of spawning,indicated by presence of post-ovulatory follicles, was detected from ovary samples taken August 28, 1986. Hydrated (H) oocytes, just prior to ovulation, were not observed in samples examined histologically until mid-September and were rare in occurrence, having been observed in 9 of 119 females from August through November. Early phase of the hydrated oocyte stage was typified by breakdown of nuclear membrane, nucleus migration towards the animal pole and formation of an oil droplet. More advanced hydrated oocytes were characterized by smooth cytoplasmic appearance and follicle collapse (preparation artifact) similar to that reported for other teleosts (Hunter and Macewicz 1985). Hydrated oocytes were present in samples as late as October 8. Mean batch fecundity for females in the mid-September collection was 1.7×10^6 hydrated eggs (range $1.0 - 2.5 \times 10^6$ eggs) and 0.7×10^6 hydrated eggs for the October 8 collection (range $0.3 - 1.0 \times 10^6$ eggs) providing evidence for decline in clutch size with time. Sampling was curtailed due to constraints on research/tagging collections after this period. Two adult females (8.7 and 9.5 kg) available in mid-November exhibited complete atresia of yolked material, providing evidence of spawning cessation (Fig. 1).

Spawning is thought to occur near dusk as evidenced by behavior of laboratory reared fish and presence of egg and larval stages observed in plankton samples (Arnold, Baily, Williams, Johnson and Lasswell 1977; Holt, Holt and Arnold 1985). Based on timing of spawning, onset of maturation and hydration may occur over 3-5 hours as hydrated eggs in early developmental phase (yolk coalescence) were evident from females collected in two purse net sets from mid-afternoon (1610 hrs. in first set, second set time approximate). Hydration in other teleosts may occur as early as 12 hours before spawning (Hunter *et al.* 1985). This may account for the rarity of hydrated oocytes among females in daytime collections and necessitates careful planning for surveys directed at batch fecundity determinations.

Because of group-synchronous recruitment patterns, batch fecundity methodology must be followed to accurately estimate total female reproductive output. Number of eggs per unit weight of ovary was significantly different at all nested source levels (Table 1). Significant differences in hydrated egg counts within ovaries may have been due to the rate of hydration among locations. Hunter *et al.* (1985) did not perceive variation in the extent of hydration as an important source of error for northern anchovies and was able to sample at a time of day when hydration was nearly complete. Histology slides prepared from six ovarian regions of each of three females (5.9, 7.1 and 11.0 kg) sampled September 13, 1985 revealed lack of divergence of propor-

tions of CA and V oocytes stages within ovaries. Vitellogenic oocytes ranged from 0.248 (n = 164/661) to 0.291 (n = 192/658) for mid regions of left and right ovarian lobes respectively. Homogeneity of overall proportions of vitellogenic oocytes supports the contention that regional differences in hydrated ova are due to rate of hydration or possible differences in vitellogenic size modes. Further work is needed to resolve this difference. However, the brief interval during which hydrated oocytes are present in red drum may require sampling of multiple locations within both ovarian lobes.

In continued work, the probability of spawning in a given time interval will be better understood and may serve as a predictive tool. This can be applied in two areas. Ovarian stage, in relation to time until spawning and as a function of gonad to body size ratio (GSI or RGI), can serve as a basis for understanding interannual variation in reproductive seasons. Secondly, accurate knowledge of the state of ovarian development can serve as a frame of reference in artificially induced spawning where standardization and replication of treatment (*i.e.*, dose level of gonadotropins) is important (Shehadeh, Madden and Thomas 1973).

ACKNOWLEDGMENTS

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SECTION III

Natural History and Larval Biology

G. Joan Holt, Editor

Contributions in Marine Science. (1988) Vol. 30:87–92.

THE USE OF STABLE ISOTOPE TRACERS IN RED DRUM (*SCIAENOPS OCELLATUS*) CULTURE

R.K. Anderson, M.L. Dickson, G.Joan Holt, D. Ritz[1] and P.L. Parker

The University of Texas at Austin
Marine Science Institute
Port Aransas, Texas 78373-1267

INTRODUCTION AND DISCUSSION

The existence of multiple stable isotopes of biologically important elements such as carbon, nitrogen, sulfur and oxygen has provided a powerful tool for tracing the flow of organic matter in ecosystems (Fry and Sherr 1984, for review). In practice, the abundance of these isotopes is measured as a ratio of the isotopic pair such as $^{13}C/^{12}C$ or $^{15}N/^{14}N$. By convention, the ratio of a sample is compared to the ratio of an arbitrarily chosen standard and expressed as a per mil (part per thousand) deviation from the ratio of the reference signified by a δ (delta) notation. Carbon isotope ratios therefore appear as $\delta^{13}C$ where:

$$\delta^{13}C = \frac{(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{std}}{(^{13}C/^{12}C)std} \times 1000$$

with similar definitions for $\delta^{15}N$, $\delta^{34}S$ and $\delta^{18}O$. Carbon isotope values are normally reported relative to a carbonate standard (PDB) and nitrogen is reported relative to air N_2.

The analytical procedure for carbon involves the complete combustion of a sample followed by cryogenic separation and purification under vacuum to obtain CO_2. The isotopic ratio of the CO_2 from the sample is determined by mass spectrometry. Similar procedures are employed for other isotopes.

The distribution of these isotopes in nature and the processes which affect that distribution are well enough understood to permit several approaches to tracer experiments in natural ecosystems and in laboratory and pond culture.

One approach is based on the nearly conservative behavior of the carbon ratio in organic matter as it passes through trophic levels. Thus a consumer's tissues assumes an integrated value of carbon which has been assimilated from all sources. Such a process lends itself to tracer experiments in which two or more isotopically distinct food sources are offered to an animal over some extended growth period. The final tissue of the animal represents a weighted average of carbon obtained from all sources. Analysis of the animal tissue after growth can provide unambiguous information about the relative assimilation of each food.

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[1] University of Tasmania, Department of Zoology, Hobart Tasmania 7001, Australia

This approach has been used with great success in determining the relative importance of natural productivity and offered feed in the pond culture of *Penaeus vannamei* (Anderson, Lawrence and Parker 1987a; Parker, Anderson and Lawrence 1988). Feeds were designed to be isotopically distinct from the natural pond biota using feedstuffs from a variety of sources which cover a wide range of $\delta^{13}C$ values. Control studies were performed in tanks where the shrimp were raised on 100% feed and in an earthen pond where the shrimp had access to the pond productivity alone. These animals were used to assess the turnover rates of tissue carbon and the rates of isotopic label acquisition. They also served as endmembers for a simple mixing model with which to interpret the results of pond trials where animals were given a choice of both food resources and obtained intermediate $\delta^{13}C$ values. Interpolation of the $\delta^{13}C$ of tissue carbon between those endmembers gives a value of relative contribution of each carbon source.

Growth trials at different stocking densities and feeding rates demonstrated that the natural production of the pond contributed from 53 to 77% to the growth of the shrimp under different conditions. It was also demonstrated that the contribution of the offered feed to the growth of the shrimp did not increase with feeding rates greater than 2.5% of body weight per day (Anderson, Lawrence and Parker 1987b).

Other experiments have been performed to evaluate a number of variables in pond culture including feed quality, stocking density, species, and feeding rate. A similar approach has been applied to penaeid larval culture to determine the timing of food selectivity (*i.e.* switching from algae to *Artemia*) as well as food preference (*Artemia versus* nematodes) when both foods are offered (Lawrence, Parker, Smith and Anderson 1987).

Such tracer experiments are directly applicable to other culture systems including that of the red drum. Preliminary work here has begun on larval development and natural history of *Sciaenops ocellatus*. Feeding experiments have been performed with newly hatched red drum in which separate tanks of a single spawn were offered either live rotifers, frozen rotifers or a commercial microcapsule feed. $\delta^{13}C$ of diets and initial animals were measured and animals were sampled from each tank twice per day from day 3 post hatch (initiation of feeding) to completion of the trial on day nine.

Growth of the larvae offered live rotifers averaged about 0.4 mm per day for the last four days of growth after an initial lag period of two days. Rates for the animals offered frozen rotifers followed the same pattern but averaged only 0.1 mm per day. Those animals offered the commercial feed exhibited no growth and most did not survive past day six post hatch as would be expected from nonfeeding starvation. The pattern of an initial lag in growth of 2-3 days after feeding commences is often observed (Lee, Holt and Arnold 1984). Figure 1 reveals that no such lag exists in the carbon isotope value of the fish which were offered either rotifer diet. The fish must have been assimilating new carbon from the initiation while the lag represented a balance of catabolism and anabolism or some growth other than an increase in standard length.

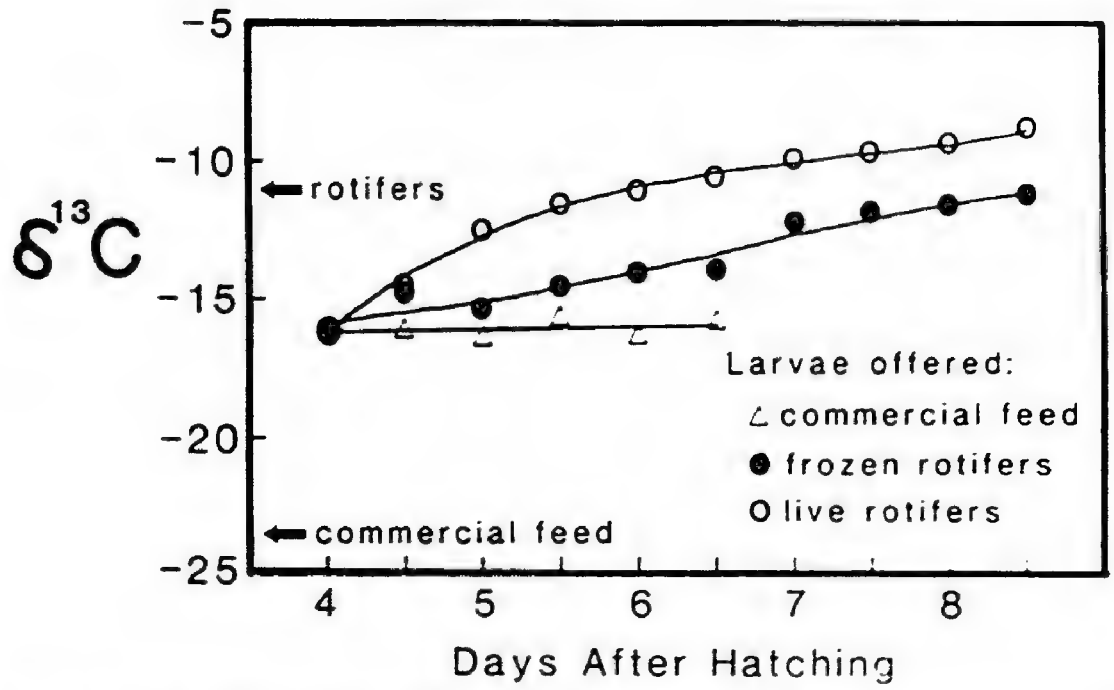

FIG. 1. $\delta^{13}C$ of red drum larvae offered live or frozen rotifers ($\delta^{13}C = -11.1‰$) or commercial feed ($\delta^{13}C = -23.2‰$).

The change in $\delta^{13}C$ of the larvae which were feeding on rotifers is a direct result of the assimilation of the rotifer carbon (− 11.1 ‰) into the tissues of the fish. The $\delta^{13}C$ of the larvae shifts toward the value of the rotifer as the original carbon of the fish is diluted by new growth. The ultimate $\delta^{13}C$ of an animal at isotopic equilibrium with its diet may be unpredictable but is usually slightly more positive (Fry and Sherr 1984). This effect is probably a complex function of the selective assimilation and differential metabolism of the various nutritional elements available within a diet (*i.e.* protein, carbohydrate and lipid). In the case of the larvae feeding on live rotifers (Fig. 1), $\delta^{13}C$ values are approaching a value about two per mil more positive than the rotifers by the conclusion of the study.

The depressed slope of the larvae feeding on frozen rotifers is a reflection of their lower growth rate. The animals which were offered the commercial feed showed no change toward the more negative value of that feed (− 23.2‰) indicating that there was no significant assimilation during the study.

We have also performed feeding experiments with red drum using stable nitrogen isotope tracers. The larval feeding switch from rotifer to *Artemia* is demonstrated in Figure 2 where the tissues of the newly feeding larvae approach the $\delta^{15}N$ of the rotifers (+ 1.5‰) in a manner completely analogous to the mixing curve of carbon isotopes. After *Artemia* are offered on day 9, the $\delta^{15}N$ of the larvae begin to reflect the addition of the more positive *Artemia* $\delta^{15}N$ (+ 12.3‰).

Nitrogen isotopes may be followed in natural foodwebs with an additional subtlety that carbon does not display. Although carbon isotope ratios are largely conservative as organic matter passes through a food chain, nitrogen isotopes tend to change by several per mil at each transfer (Miyake and Wada 1967; Steele and Daniel 1978; Macko, Lee and Parker 1982). Top carnivores in a marine food web may display $\delta^{15}N$ values of approximately + 15‰ while

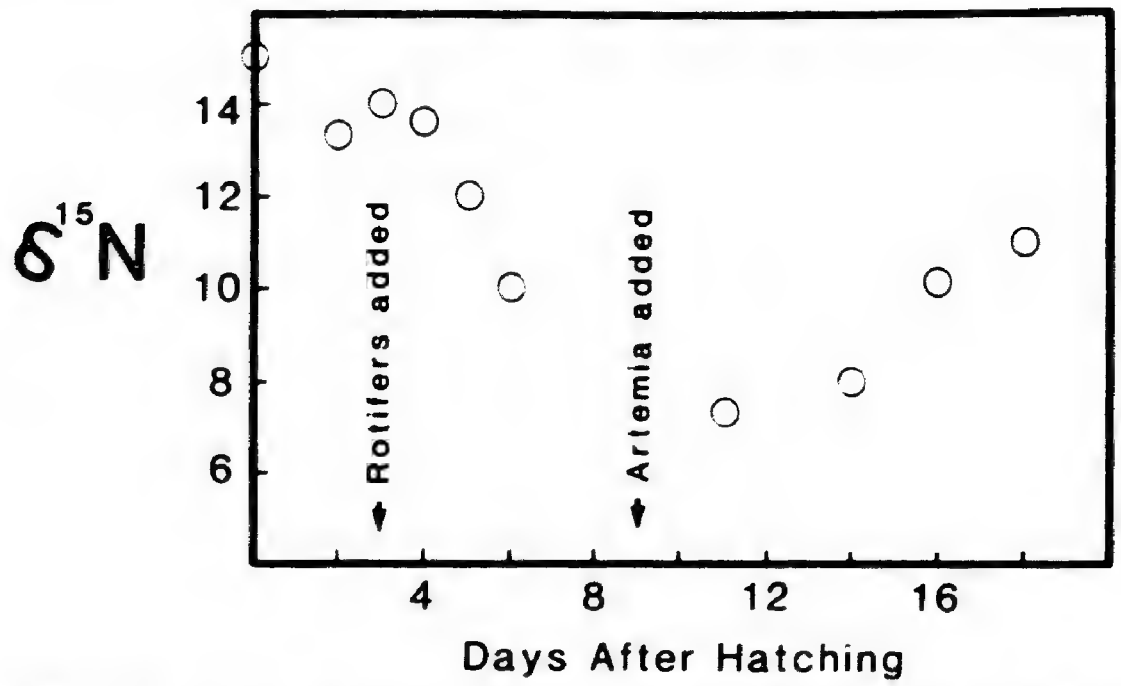

FIG. 2. $\delta^{15}N$ of developing red drum larvae as offered feed changes from live rotifers ($\delta^{15}N$ = + 1.5‰) to live *Artemia* ($\delta^{15}N$ = + 12.3‰).

herbivores and secondary consumers will appear with values more like +5 and +10‰ respectively. Thus surveys of natural abundances of nitrogen isotopes tend to provide additional information about trophic structure to which carbon variations may be less sensitive. We will be examining the natural abundances of carbon and nitrogen in red drum, flounder and trout in the local estuaries, bays and gulf to elucidate details of their feeding strategies and resource utilization.

CONCLUSION

Stable isotope tracers may also be applied to more fundamental questions of nutrition and metabolism by differential labeling of components within the feed. Studies are underway with *P. vannamei* to determine the nutritional sources of lipids (especially fatty acids) in pond cultured shrimp and how it may differ by changing the lipid quality (source) in the offered feed. The possibilities for similar nutritional studies with red drum are obvious. The studies reported here with red drum represent an initial effort which demonstrates the suitability and appropriateness of these methods for investigations concerned with its culture, natural history and fundamental nutrition.

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Contributions in Marine Science. (1988) Supp. to Vol. 30:93–98.

GROWTH RATES AND VALIDATION OF AGE ESTIMATES OF RED DRUM, *SCIAENOPS OCELLATUS*, IN A LOUISIANA SALT MARSH IMPOUNDMENT

Daniel W. Beckman, Gary R. Fitzhugh, and Charles A. Wilson

Coastal Fisheries Institute
Center for Wetland Resources
Louisiana State University
Baton Rouge, LA 70803-7503

ABSTRACT

The objectives of this study were to determine growth rates and validate age estimates of red drum in a coastal Louisiana impoundment. One hundred and eighty six yearling red drum were tagged and released into the impoundment after injection of selected individuals with varying concentrations of oxytetracycline to incorporate a fluorescent marker into their otoliths.

Thirty individuals were recaptured over the next 20 months. Nineteen were retained for analysis. A low growth rate was observed for initial recaptures; however, mean growth rate of red drum at large for more than 200 days was 0.58 mm/d and 4.2 g/d.

Transverse sections of otoliths (sagittae) from tetracycline injected returns exhibited a fluorescent mark under ultraviolet light corresponding to the date of release. Subsequent annuli were laid down in sagittae at the rate of one per year; opaque and translucent zones corresponding to winter-spring and summer-fall growth, respectively. Thus yearly age estimates based on annuli counts were validated up to approximately 2.5 years in age.

INTRODUCTION

Estimates of growth rates of juvenile red drum *Sciaenops ocellatus* have been primarily indirect, based on changes in modal or mean lengths (Pearson 1928, Roessler 1970, Bass and Avault 1975, Theiling and Loyacano 1976, Wakeman and Ramsey 1985). Such estimates are imprecise as they are confounded by recruitment, movements, mortality, sampling bias, and variation in individual growth rates. A direct measure of growth can be obtained through mark-recapture studies. Knowledge of growth rates of juvenile red drum is essential for aquaculture feasibility studies. Simmons and Breuer (1962) obtained growth rates of immature tagged-recaptured red drum in Texas coastal waters, otherwise no direct growth rate determinations are available.

Ages of red drum have been estimated by following modes in length-frequency distributions and counting scale annuli (Pearson 1928, Wakeman

and Ramsey 1985). Age estimates based on changes in length-frequency distributions are confounded by overlapping year classes due to extended spawning seasons and variable growth. Age estimates from scales have not been validated as is necessary for understanding the temporal meaning of the annuli (Beamish and McFarlane 1983). Theiling and Loyacano (1976) utilized counts of otolith annuli for age estimations; however, they did not provide complete validation.

Direct validation of age estimations requires fish of known age or mark-recapture studies in which a time marker is incorporated into the hardpart used for age estimates. Tetracycline has been injected into fish to impart a fluorescent mark on hardparts such as spines (Tucker 1985), vertebrae (Holden and Vince 1973), and otoliths (Wild and Foreman 1980; Beamish, McFarlane and Chilton 1983) to validate age estimates. The objectives of this study were to directly measure growth rates for immature red drum released into a salt marsh impoundment, determine the time of first annulus formation in otoliths (sagittae), and directly validate age estimation techniques.

MATERIALS AND METHODS

One hundred and eighty six red drum were captured by hook-and-line in a southeast Louisiana salt marsh on August 8, 1985 and immediately placed into holding pens. Following weighing and measurement (total length) three groups of red drum were tagged with the following:

1) Floy FT-4 cinch-up tag [N = 63],
2) Floy FT-68 anchor tag [N = 62], and
3) Floy belly tag with 8 mm diameter laminated disk [N = 61].

Within each experimental tag group, three tetracycline dosage levels were administered interperitoneally by syringe to subgroups of approximately 20 fish at 0 (sham injected control), 50, and 100 mg/kg body weight using Liquamycin LA-200 with 200 mg/L oxytetracycline base. Fish were released into a 1,000 hectare salt marsh impoundment. Total time from capture to release was less than one hour.

For recaptured fish, date of capture, fork length and weight were recorded as available. Sagittae were removed and embedded in an epoxy resin medium (Spurr 1969). Transverse sections (0.7 mm thick) were made through the core of the left sagitta using a Buehler Isomet low-speed saw and observed under a compound microscope with incident ultraviolet (UV) light for detection of fluorescent marks. Observations of annuli formed after incorporation of the fluorescent tetracycline mark were made under transmitted light at 40X magnification and compared to the time at large. Annuli (opaque zones) were counted medial to the core of the sagitta, the region of the most regular growth.

RESULTS AND DISCUSSION

High waters as a result of hurricane Juan in October, 1985 resulted in the release of some of the tagged red drum from the impoundment. Eighteen individuals were recaptured inside the impoundment after being at large from 16 to 610 days. Twelve recaptures were reported from outside the impoundment, however only 1 individual, at large for 140 days, was returned.

Fish returned from inside the impoundment were divided into 2 groups:

1) Growth of four individuals at large for 16 to 76 days ranged from 0.0 to 0.41 mm/d ($\bar{x}$ = 0.17, SD = 0.20), and −1.4 to 1.2 g/d (x = 0.03, SD 1.28).

2) Growth rate of individuals at large for 348 to 610 days (N = 14) ranged from 0.40 to 0.72 mm/d ($\bar{x}$ = 0.57, SD = 0.11), and from 3.0 to 5.5 g/d (x = 4.2, SD = 0.87, N = 8 weights).

Growth rate of the return from outside the impoundment was 0.48 mm/d (weight not available). The growth of each red drum recaptured is shown in Figure 1.

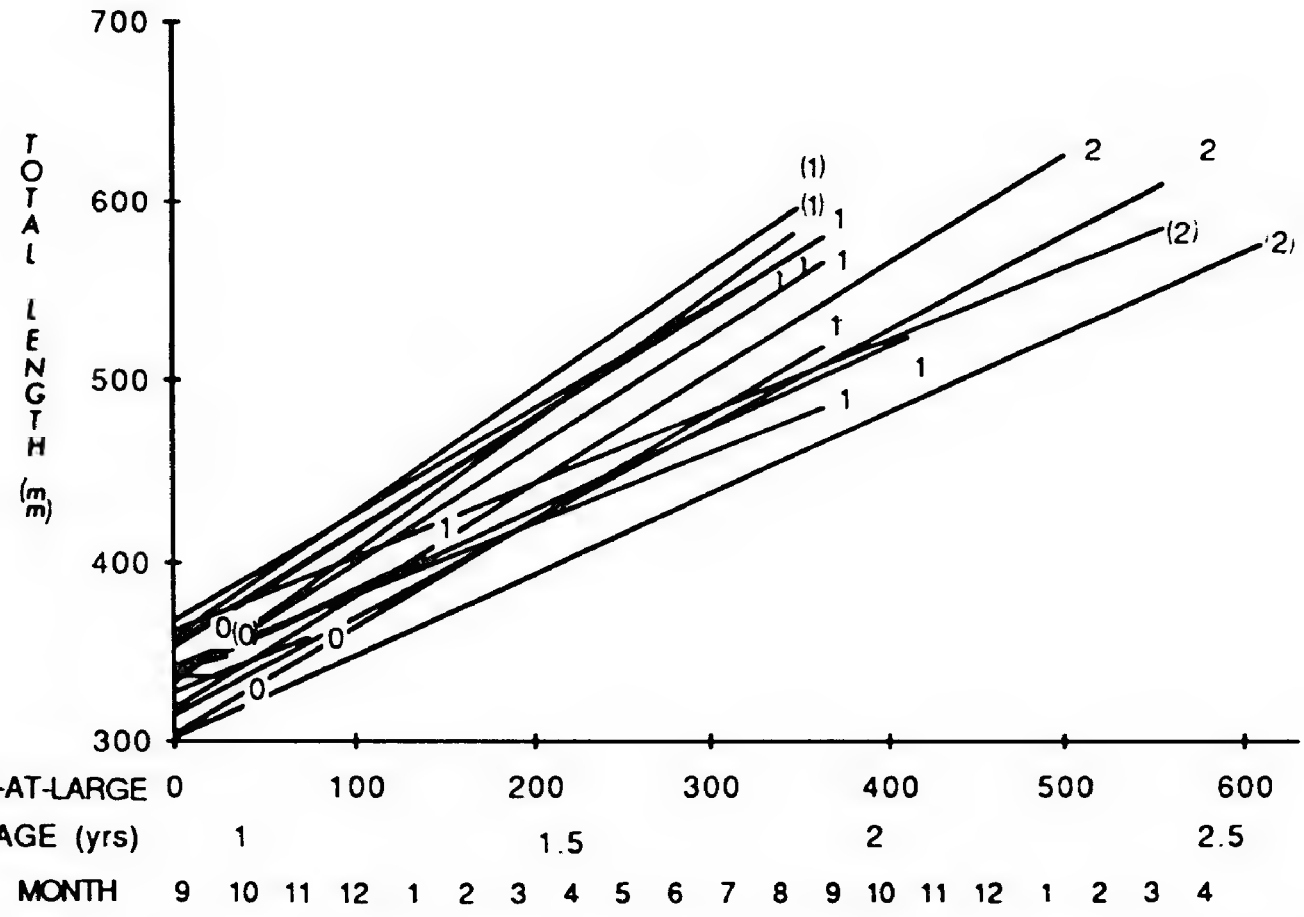


FIG. 1. Growth of tagged-recaptured red drum. Lines depict the change in length of individual fish from date of release (0 days-at-large) to date of recapture. Numbers adjacent to lines indicate the number of annuli in sagittae at time of recapture. Numbers in parentheses correspond to fish which were not injected with tetracycline.

Growth rates may have been reduced in the early (Group 1) returns due to initial stress due to handling and injection. If this were true then all growth rates would be reduced from that expected for unmarked fish. An alternative explanation is that the summer-fall period when Group 1 fish were at large is one of naturally lower growth rates. The tetracycline did not appear to affect growth of fish in Group 2, as the mean growth of returns from the 0, 50, and 100 mg/kg injection groups were 0.57 mm/d (SD = 0.16, N = 4), 0.56 mm/d (SD = 0.10, N = 6), and 0.59 mm/d (SD = 0.04, N = 3) respectively. Though fewer returns were received from the higher injection dosage (There were 11, 11, and 6 returns for the 0, 50, and 100 mg/kg dosage levels, respectively), the significance of these differences could not be ascertained. A larger number of returns would be necessary to determine the effect of tagging and injection.

Growth rates reported in this study were generally greater than those reported by Wakeman and Ramsey (1985) (0.45 mm/d) calculated from

changes in length distribution modes for one-year-old red drum in Louisiana. Our data compared closely to Pearson's (1928) calculation of 0.54 mm/d from changes in average length of red drum from one to 1.5 years age in Texas, and Simmons and Breuer's (1962) reported 0.59 mm/d for one-year-olds from mark-recaptures in Texas. This suggests that the red drum in our study exhibited natural growth rates regardless of injection, tagging, and retention in the impoundment.

All recaptured red drum which had been injected with tetracycline exhibited a fluorescent mark in the sagittae under UV light (Fig. 2). The amount of growth in the sagitta subsequent to the mark increased with time at large. Uninjected fish did not exhibit a fluorescent mark.

Annuli were not observed in sagittae of individuals recaptured from September through November, 1985. All tetracycline injected red drum recaptured between January and November, 1986 had one opaque zone (annulus) between the fluorescent mark and the edge of the sagitta. Sagittae from individuals recaptured from January through April 1987 had two annuli following the fluorescent mark. Sagittae from control group returns (not injected with tetracycline) exhibited identical patterns of annulus formation as returns from injection groups (Fig. 1). There was 100% agreement among three readers for all annulus counts, made without knowledge of the sample source. These data show that annuli were formed once per year, with a winter-spring opaque zone and a summer-fall translucent zone suggested. Further studies are necessary to determine the exact timing of opaque and translucent zone formation in red drum sagittae.

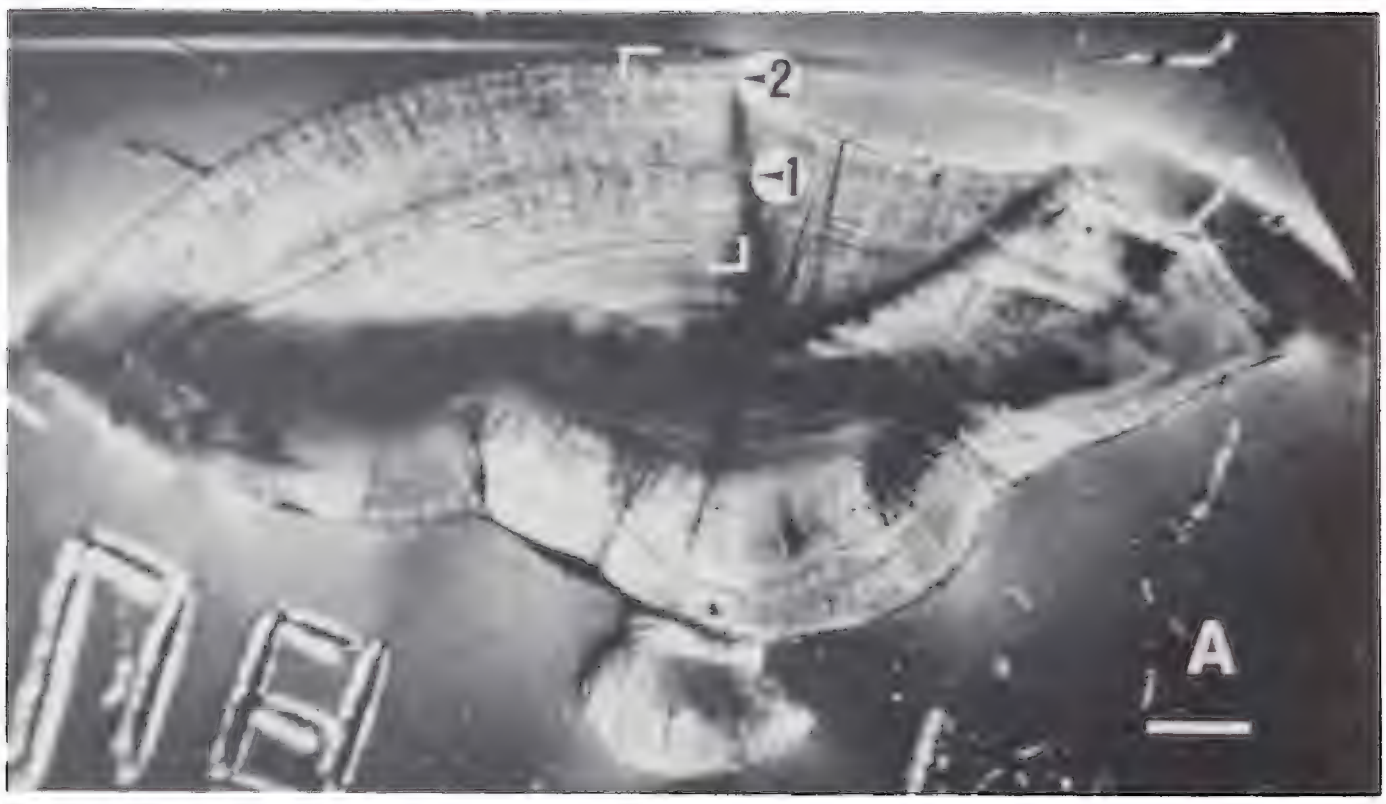
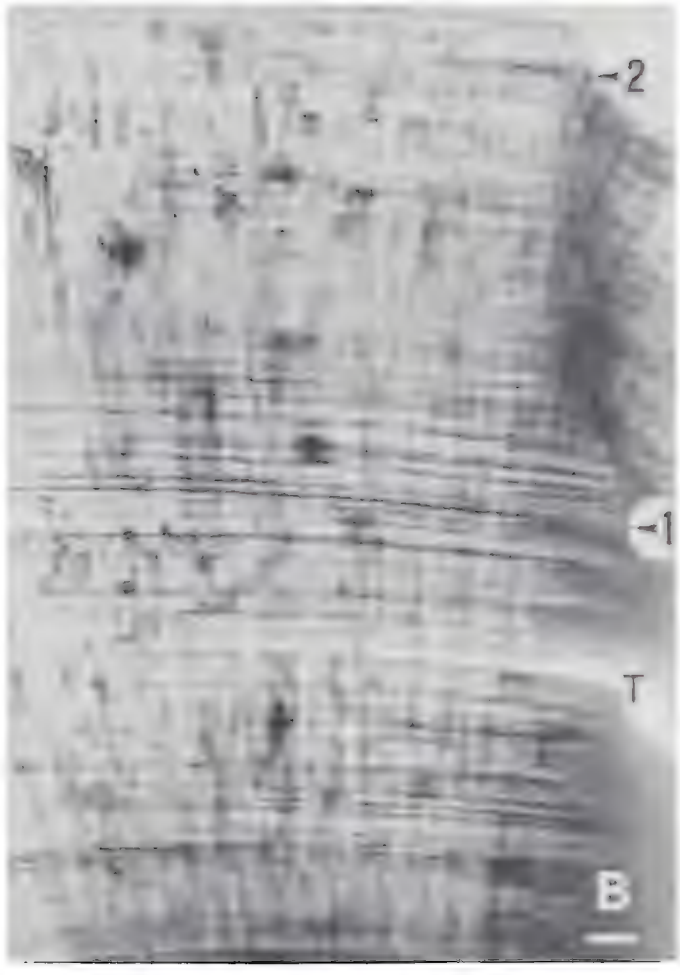


FIG. 2. Transverse section from a sagitta of a red drum injected with 100 mg tetracycline per kg body weight August 28, 1985, and recaptured March 7, 1987 (556 days-at-large).
A) is entire section under bright field light. Bar = 1 mm.

Red drum spawning is concentrated from August to October in the northern Gulf of Mexico, with peak spawning around September-October (Simmons and Breuer 1962), and individuals grow to a total length of approximately 300-350 mm during their first year (Pearson 1928, Peters and McMichael 1987). Therefore red drum in this study were approximately 11 months old when released in August, assuming birth around the first of October the year prior to release. Based on this information, ages were determined from counts of annuli in the sagittae (Fig. 1). Formation of the first annulus began at approximately 15 months for fish spawned in October, and was followed by formation of a second annulus 12 months later.

CONCLUSIONS

In summary, average growth of one and two year old red drum in a Louisiana salt marsh impoundment was 0.57 mm/d and was similar to growth rates reported in other studies for unimpounded red drum. Counts of annuli in transverse sections of sagittae can be used to estimate ages of red drum for at least the first 2.5 years.



B) is area indicated by brackets in (A) under bright field plus ultraviolet light. Bar = 0.1 mm. Fluorescent tetracycline mark is indicated (T). Numbers indicate center of opaque zones (annuli).

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A PROCEDURE FOR IDENTIFYING SCIAENID EGGS

S.A. Holt, G. Joan Holt and L. Young-Abel

The University of Texas at Austin
Marine Science Institute
Port Aransas, Texas 78373-1267

ABSTRACT

Eggs of members of the family sciaenidae are not distinguishable morphologically but one-day old yolk-sac larvae can be separated based on pigmentation. This identification procedure requires an unpreserved ichthyoplankton tow from which live eggs are picked with a pipette and sorted by size. Sixteen to twenty hours after hatching, the larvae can be identified using the accompanying illustrations. Eggs of a given size in preserved samples are identified to species based on the identification of the larvae which hatched from eggs of like size from concurrently obtained unpreserved samples.

This procedure requires little special equipment and can even be done on shipboard. Although this process is rather time consuming and is not practical for large surveys it has proven useful for our studies and is the only reliable procedure for accurate identification of sciaenid eggs.

INTRODUCTION

Although the eggs of a few marine fish species are recognizable, most fish eggs from ichthyoplankton samples remain classified as "fish eggs." Joseph, Massmann and Norcorss (1964) states that "Identification of pelagic eggs of marine fish has proven to be a frustrating task. Attempts to work with eggs of the drum family, Sciaenidae, have been especially unrewarding." The situation has remained essentially unchanged since that time, especially in regard to sciaenid eggs. Joseph *et al.* (1964) cited extant descriptions of three species of Atlantic and Gulf coast sciaenids, weakfish (*Cynoscion regalis*), northern kingfish (*Menticirrhus saxatilis*), and silver perch (*Bairdiella chrysoura*), plus their description of black drum (*Pogonias cromis*) as the only sciaenids for which the eggs were described. Although eggs of spotted seatrout (*Cynoscion nebulosus*) (Fable, Williams and Arnold 1978), and red drum (*Sciaenops ocellatus*) (Holt, Johnson, Arnold, Fable and Williams 1981a) have subsequently been described, the morphological similarity and overlap in reported sizes have continued to prevent reliable identification of sciaenid eggs.

A procedure for identifying eggs of most common sciaenids which spawn in the estuarine and nearshore areas of the northwestern Gulf of Mexico has been developed. Sciaenid eggs have been hatched and reared to an identifiable stage using larval rearing facilities at the Fisheries and Mariculture Laboratory of the University of Texas Marine Science Institute. It was found that one-day old larvae can be identified and this paper describes the techniques and presents a guide for separating seven species of sciaenids.

METHODS

This identification procedure requires an extra ichthyoplankton tow which is kept unpreserved in a bucket. As soon as possible, the live eggs are picked from the sample with a pipette under a dissecting scope and sorted by size. The eggs are put in a covered petri dish filled about two thirds full with water from the sample bucket, filtered through 60 μm mesh. A cover is necessary to reduce evaporation and the resultant high salinity. Only 12 to 15 eggs of each size-class are needed since high egg densities will quickly produce water quality problems. All sciaenids described herein spawn near sunset and eggs begin to hatch 16 to 22 hours after spawning in 25-27°C (Holt, Holt and Arnold 1985). This means that all eggs will hatch around 1500 to 1900 h so that egg handling must be complete by 1300 to 1400 h. The next morning, approximately 15 hr after hatching, larvae are observed on a well-slide under the dissecting scope and identified using the accompanying illustrations. With practice, larvae can often be identified without removing them from the petri dish.

Typically, all the larvae from one size-class of egg will be the same species, and therefore sciaenid eggs of corresponding size in the preserved samples can be assigned to that species. When more than one species is identified from one egg size-class, we determine the proportion that each species constitutes in the live sample, and assign species identifications to the preserved eggs in the same proportion.

RESULTS AND DISCUSSION

This is actually a guide to one-day-old larvae which, in conjunction with egg size measurements and known spawning dates (Table 1), has proven

TABLE 1

Minimum and maximum egg diameter, temperature (°C) and salinity (ppt) ranges, and extreme dated eggs were collected for each species.

	Egg size (mm)		Temperature		Salinity		Date		No. of
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Observations
Atlantic croaker	0 72	0 75	23 5	26 0	30	31	25 Oct	14 Dec	2
Black drum	0 90	1 20	18 0	25 0	26	36	6 Mar	22 Apr	9
Menbcirrhus sp	0 63	0 87	18 5	29 5	25	37	20 Mar	3 Oct	39
Red drum	0 83	1 00	24 0	31 0	28	35	20 Aug	10 Oct	23
Sand seatrout	0 67	0 90	24 5	29 0	27	37	12 May	30 Aug	12
Silver perch	0 59	0 82	22 0	30 0	25	36	31 Mar	10 Sep	28
Spotted seatrout	0 60	0 85	22 0	31 0	19	36	31 Mar	1 Oct	36

reliable in providing egg identifications of seven common sciaenids. All seven species were reared from eggs using techniques described for red drum (Holt, Godbout and Arnold 1981b). As an out-growth of this work we developed a method for identifying wild collected eggs which could be used in any lab and did not entail the time consuming task of rearing and feeding larvae.

Initial positive identification of eggs was accomplished in two ways:

1) adult fish brought into the laboratory were induced to spawn either by temperature—photoperiod manipulations (Arnold 1978) or hormone injections, or

2) wild-collected eggs were hatched and reared until they could be identified using published literature.

Red drum, spotted seatrout, Atlantic croaker and sand seatrout were spawned in the lab. This paper presents the first published information on sand seatrout eggs and yolk-sac larvae.

Eggs of sciaenids described herein are morphologically similar and share the following characteristics: positively buoyant (at least at the salinities in which they were spawned); semitransparent; generally < 1mm in diameter (Table 1); possess one oil globule in later stages of egg development; both oil globule and embryo profusely pigmented, primarily with yellow chromatophores rather than black ones. While these characteristics are universal to sciaenids we have seen, they do not serve to uniquely separate them from other families. Eggs of some Scombridae, Sparidae, Stromateidae, Haemulidae, and probably others, have similar characteristics (Joseph et al. 1964). We have had particular difficulty distinguishing eggs of pigfish (*Orthopristis chrysoptera*, Haemulidae), from those of sciaenids; however, the oil globule in pigfish is in the anterior portion of the yolk sac in one-day-old larvae, whereas in sciaenids it is in the posterior portion of the yolk-sac. Because of the similarity of eggs among species and even families, positive identification of eggs relies on characteristics of hatched larvae.

The one-day old larvae described below are basically similar. All are preflexion larvae with unpigmented eyes and a large yolk-sac. Pigmentation is composed largely of yellow chromatophores, and black pigments are rare. Viewed with transmitted light on a stereoscope, these pigments appear dark brown but viewed with reflected light they are bright golden-yellow. All references to pigment refer to these yellow pigments rather than black. Illustrations are presented for seven species: black drum, spotted seatrout, sand seatrout, Atlantic croaker, red drum, silver perch, and *Menticirrhus* spp. (Fig. 1) Variability in pigment pattern among individuals within each species was small compared to differences among species. A composite drawing emphasizing salient features of each species was prepared based on camera lucida drawings of anesthetized larvae.

Species Descriptions

Black drum

Black drum have three chromatophore bands. The first is immediately posterior to the vent, the second and most distinct band is located midway between the vent and tip of the notochord, and the third band is three quarters of the way between the anus and notochord tip. All three bands consist of distinct dendritic chromatophores. Yellow chromatophores are aggregated above the abdomen, on the nape and in a band from the posterior portion of the eye to the top of the head. Small pigment spots outline the posterior end of the notochord.

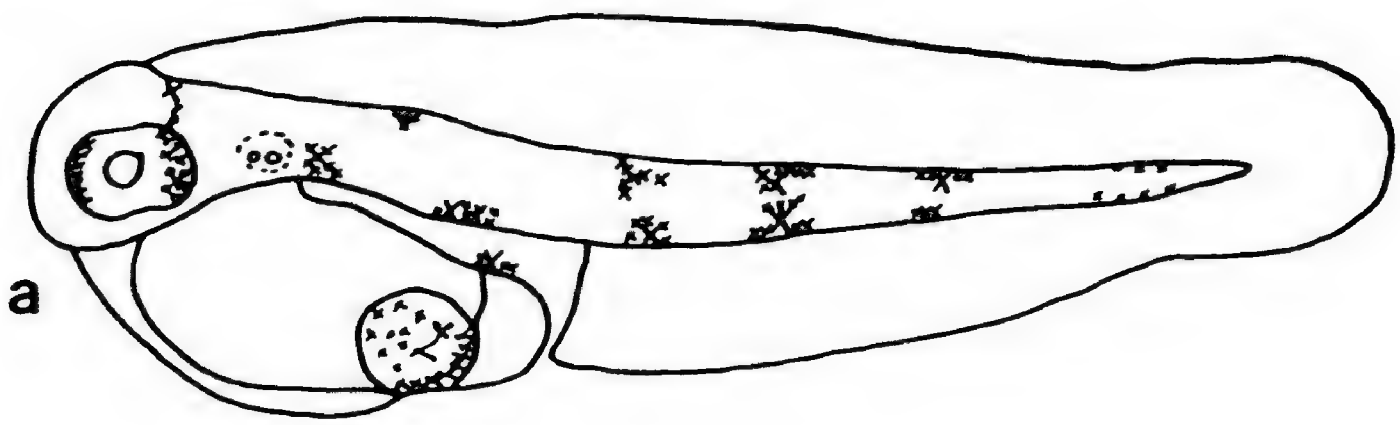
Spotted seatrout

Spotted seatrout have two chromatophore bands, one at the vent and the other midway between the vent and tip of the notochord. These bands are

quite broad and consist of web-like pigmentation. On many larvae the bands converge and pigment essentially covers the posterior two-thirds of the larva. Yellow chromatophores are aggregated behind the eye, on the head in front of the eye, on the oil globule, and dorsally above the yolk-sac. The aggregation of pigment above the yolk-sac sometimes forms a band. Small pigment spots outline the notochord tip.

Sand seatrout

Sand seatrout have two chromatophore bands, one at the vent and the other midway between the vent and tip of the notochord, the second one being the



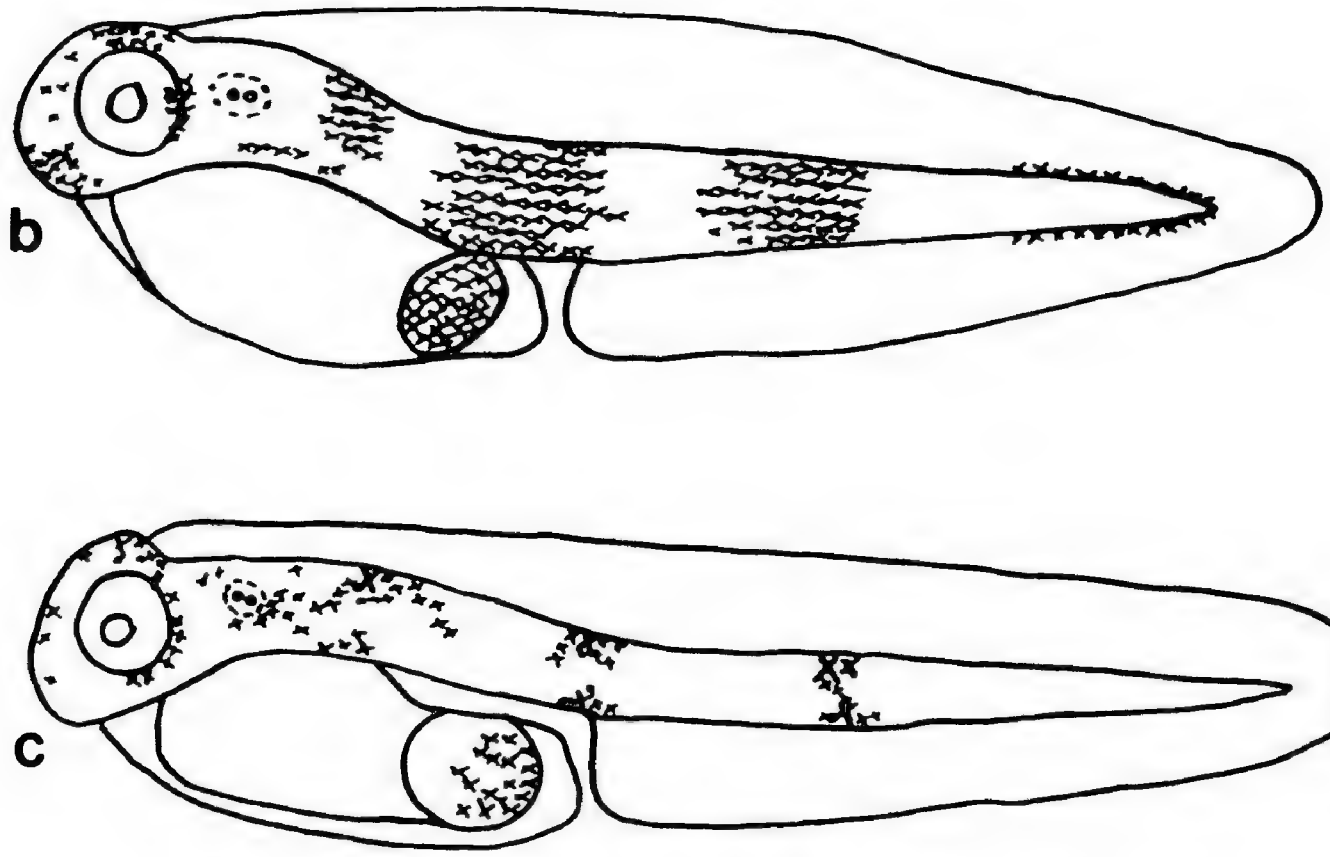
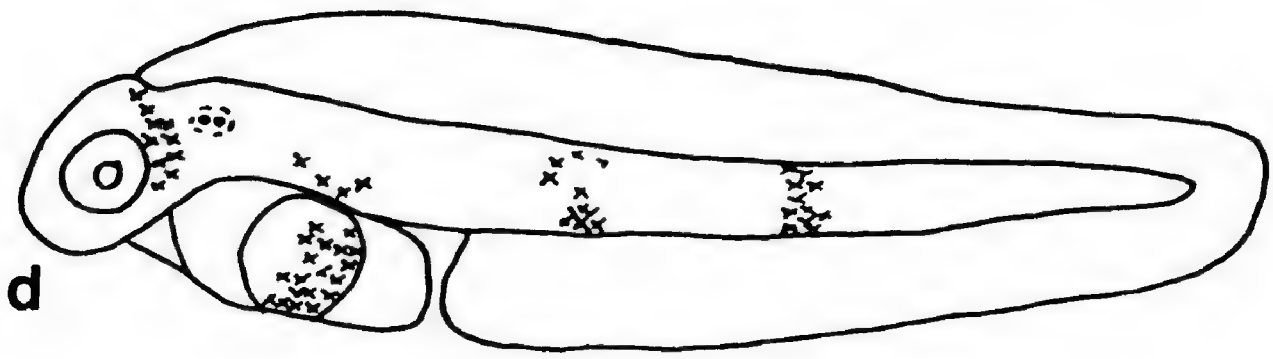
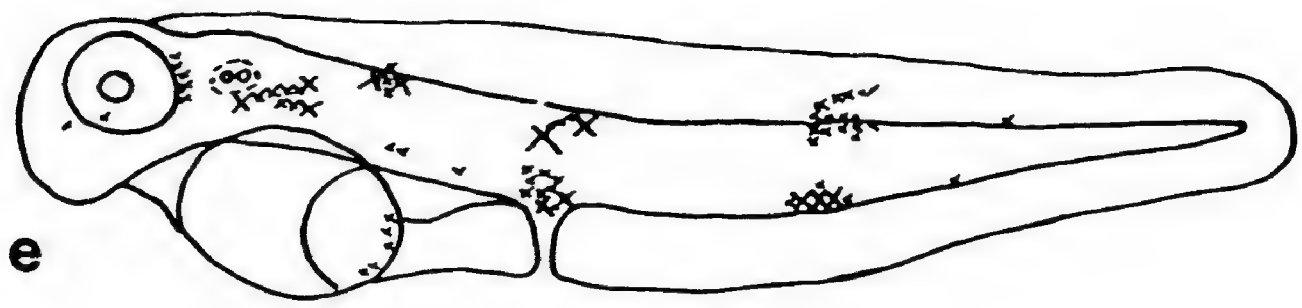
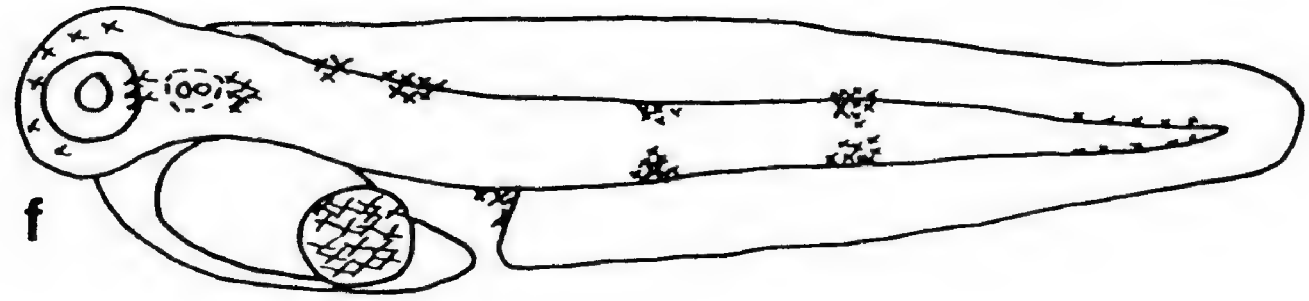
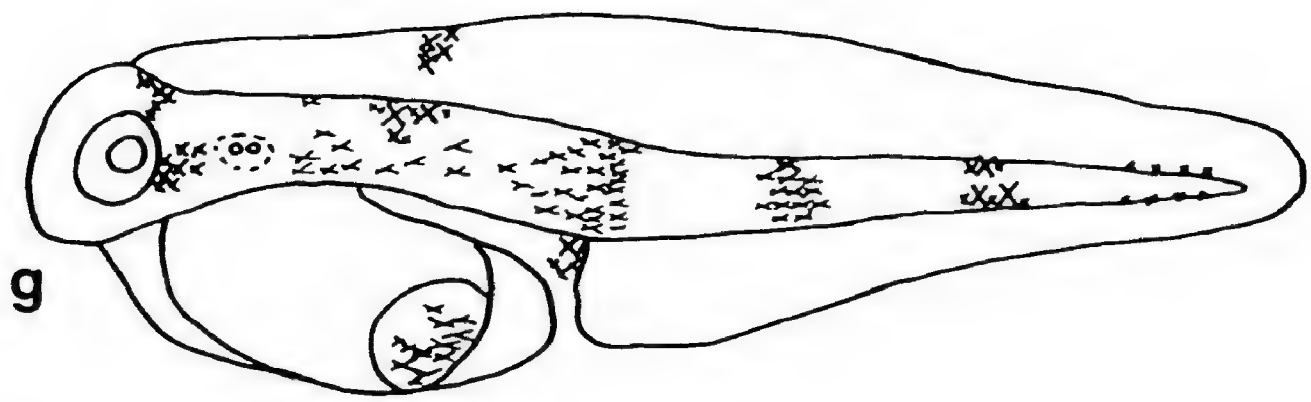


FIG. 1 Yolk-sac larvae of seven species of sciaenids 16 to 20 h after hatching: a) black drum (*Pogonias cromis*) 2.61 mm, b) spotted seatrout (*Cynoscion nebulosus*) 2.05 mm, c) sand seatrout (*Cynoscion arenarius*) 2.19 mm, d) Atlantic croaker (*Micropogonias undulatus*) 1.9 mm, e) red drum (*Sciaenops ocellatus*) 2.43 mm, f) silver perch (*Bairdiella chrysoura*) 2.20 mm,









and g) *Menticirrhus* spp. 2.13 mm. Illustrations are of anesthetized larvae. All pigmentation consist of golden-yellow rather than black chromatophores. Sizes are mean notochord lengths of three individuals used to make the composite illustrations.

most distinct. Yellow chromatophores are aggregated on top of head and behind the eye. There is no pigmentation between the second band and the tip of the notochord.

Atlantic croaker

Atlantic croaker have two chromatophore bands. One slightly posterior to the vent, and the other midway between the vent and notochord. The second band is the most distinct and consists of streaky yellow pigment. The first band contracts as the larva ages to form a ventral pigment spot posterior to the vent within 24 h after hatching. Yellow chromatophores are aggregated behind the eye. There is no pigmentation between the second band and the tip of the notochord.

Red drum

Red drum have two chromatophore bands, one at the vent and the other midway between the vent and tip of the notochord, the first one being the most distinct. The branching chromatophores of the second band often extend into the dorsal finfold. Yellow chromatophores are aggregated behind the auditory vesicle and on the dorsal midline above the oil globule. Two small chromatophores, one dorsal and one ventral, lie between the second band and the tip of the notochord.

Silver perch

Silver perch have two chromatophore bands, the first is one-third and the other is two-thirds of the way between the vent and tip of the notochord. The second band is the most distinct. Yellow chromatophores aggregate behind the auditory vesicle, on the dorsal body surface above the yolk-sac, and on the posterior part of the vent. Small pigment spots outline the tip of the notochord.

Menticirrhus spp.

There is probably more than one species of *Menticirrhus* collected in our samples but no consistent pigmentation differences have been found. All the *Menticirrhus* have three chromatophore bands. The first is immediately posterior to the vent or above the posterior edge of the vent, the second very distinct band lies midway between the vent and tip of the notochord, and the third band is three quarters the way between the vent and the notochord tip. The first two bands consist of streaky yellow pigment. Yellow chromatophores aggregate behind the eye, on the nape and outline the tip of the notochord. Streaks of yellow pigment are also scattered on the body above the yolk-sac. Yellow chromatophores are aggregated in the dorsal finfold above the yolk-sac.

Species Separation

The temporal pattern of spawning will aid in identification; inclusive spawning dates for the seven species collected in our area are given in Table 1. Additionally we have included the minimum and maximum temperatures

and salinities associated with eggs of each species. Due to overlap in spawning period and egg sizes, ultimate identification of eggs depends on identification of the hatched larvae.

The important features distinguishing the larvae described herein are given below. Sand seatrout, red drum, silver perch, and Atlantic croaker are fairly similar. All have two more or less distinct bands of yellow chromatophores above or posterior to the vent and an aggregation of pigment on the nape and behind the auditory vesicle. Sand seatrout, unlike the others, has a distinct aggregation of chromatophores medially on the dorsal surface of the head, thus separating it from the very similar red drum. Silver perch and Atlantic croaker have pigment bands which are more posterior to the vent than those of either red drum or sand seatrout. Silver perch has yellow chromatophores outlining the tip of the notochord and on the nape, whereas these pigments are lacking in Atlantic croaker. Black drum, and *Menticirrhus* spp. unlike the other sciaenids, have three pigment bands posterior to the vent and a distinct pigment band from the eye to the top of the head. *Menticirrhus* spp. are distinguished by pigmentation in the finfold above the nape and streaky pigmentation on the body above the yolk-sac. Spotted seatrout are easily recognized by the streaky pigmentation that covers most of the body.

Example Data Set

The following is an example of our use of this technique in a survey of red drum eggs in Aransas Bay. Surface plankton samples were taken on three occasions at four sites in the Aransas Pass and Aransas Bay during red drum spawning season. A live sample, as well as two preserved samples, were obtained from each site and returned to the laboratory. The suite of four samples took about five hours to collect and the live material was back in the laboratory by 1300 or 1400 h, with all sciaenid eggs in late embryo stage. (Live sample containers could be kept in an ice chest with a small amount of ice to slow egg development to insure that eggs do not hatch.) Live eggs were picked and sorted into size-classes. In this case only the egg size-classes which produced red drum larvae were recorded, but eggs of other species could be separated and identified for a general survey of sciaenids. These data were then applied to the counts from the preserved quantitative samples to determine density of red drum eggs. Table 2 shows density of all sciaenid eggs taken from the preserved samples with red drum eggs (based on identifying one-day-old larvae hatched from the live samples) underlined. Egg sizes which did not produce red drum larvae (and not identified in this case) are not underlined.

While egg sizes were consistent among stations for a given date, egg sizes changed during the study. It can be seen from Table 2 that size ranges overlap and size alone was inadequate to identify the species in question. Therefore, correct identifications were made only by using this egg hatching method. Problems with overlapping egg size-classes are even more complex in

TABLE 2

Number of sciaenid eggs converted to no. per 100 M^{-3} of each size taken in preserved samples from each station and replicate, underlined numbers are egg size-classes which were identified as red drum based on one-day-old larvae from hatched eggs.

			Egg Diameter (mm)											
Date	Station	Rep	0.55	0.59	0.63	0.67	0.71	0.75	0.78	0.82	0.86	0.90	0.94	0.98	Red Drum
11 Sept 1984	1	1		131.72	175.62	307.34	175.62			439.06	2019.69	834.22			3292.97
11 Sept 1984	1	2	227.34	410.30	91.18	45.59	136.77			91.18	2005.91	182.36			2279.44
11 Sept 1984	1	3		113.83		142.19				256.12	1508.28	256.12			2020.52
11 Sept 1984	2	1			35.67	160.52				17.84	107.01	89.18			214.02
11 Sept 1984	2	2	18.68	579.19	56.05	261.57	205.52			74.73	74.73				149.47
11 Sept 1984	3	1													
11 Sept 1984	3	2													
11 Sept 1984	4	1								3.90	1.95				
11 Sept 1984	4	2													
28 Sept 1984	2	1		25.58	159.50	266.83	584.83	186.38			279.12	531.66	132.92		943.70
28 Sept 1984	2	2		3.80	45.55	15.18	121.48	18.98		7.59	87.31	79.72	15.18		189.81
28 Sept 1984	2	4			34.66	225.30	69.32				17.33				17.33
28 Sept 1984	2	5			68.76	34.38									
28 Sept 1984	3	1													
28 Sept 1984	3	2													
28 Sept 1984	4	1													
28 Sept 1984	4	2													
3 Oct 1984	1	1						39.39	13.13		13.13	122.56	161.95	13.13	357.76
3 Oct 1984	1	2					5.40	59.41	5.40		16.20	167.44	135.03	10.80	310.77
3 Oct 1984	2	1			7.35	7.35	88.19	36.54	117.59	44.10	66.15	360.12	360.12	29.40	329.48
3 Oct 1984	2	2					41.18	123.53	74.12	8.24		205.88	370.58	41.18	815.79
3 Oct 1984	3	1													
3 Oct 1984	3	2													
3 Oct 1984	4	1													
3 Oct 1984	4	2													

[1] Sciaenid eggs from this station were not red drum

plankton samples collected April-August because of the large number of sciaenids spawning then (Table 1). Note particularly the overlap in size among eggs from sand seatrout, silver perch, *Menticirrhus* spp. and spotted seatrout. The egg hatching, one-day-old identification technique described here is the only reliable way to positively identify eggs and to obtain quantitative egg data.

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FINE-SCALE SPATIAL DISTRIBUTION OF RED DRUM, *SCIAENOPS OCELLATUS*, LARVAE

Richard F. Shaw, David L. Drullinger, Kenneth A. Edds[1] and Deborah L. Leffler[1]

Coastal Fisheries Institute
Center for Wetland Resources
Louisiana State University
Baton Rouge, Louisiana 70803

ABSTRACT

Fine-scale (1 m to 1 km) spatial distribution was determined for larval red drum collected during their peak abundance in 1986 off the Louisiana-Mississippi barrier islands. Surface ichthyoplankton tows were made on 6 two-day cruises between 5 August and 19 November 1986, but significant densities were encountered only during late September. Tows were taken during two consecutive nights and the interim day, around a set of three, centrally-located, windowshade current drogues in an attempt to repeatedly sample the same water mass over time. Plankton stations were 0.23 km (0.13 nautical miles) apart. During late September substantial spatial variability in larval red drum densities was found on a scale of 230-700 m within a 30 h period. During that same 30 h period, values for David and Moore's index of clumping and Lloyd's index of patchiness calculated from station densities ranged from 8.61 to 32.73 and 1.21 to 2.32, respectively, indicating an overdispersed distribution and at least a moderate degree of patchiness over the small spatial scale examined. The distribution of red drum eggs and larvae may be particularly patchy because of the adults' aggregative spawning behavior which is nocturnal, synchronous and of short-duration.

INTRODUCTION

Spatial heterogeneity is an important factor in virtually every ecosystem. Patchiness is considered to be ubiquitous and has been observed at virtually every temporal and spatial scale investigated (Levin 1978). Aggregated or clumped distributions were formerly considered a nuisance in plankton sampling or statistical analysis but are currently recognized as a possible adaptive strategy suggesting growth, mortality and recruitment implications (Hewitt 1981, McGurk 1986). In the horizontal plane, "plankton patchiness" usually refers to spatial variability at scales between 10 m and 100 km, while vertical variability can be observed at scales between 0.1 m and 50 m (Mackas, Denman and Abbott 1985). Patch dimension has been further categorized into microscale, 1-100 cm, fine- or small-scale, 1-1000 m, and coarse or mesoscale, 1-1000 km (Houde 1982).

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[1] Department of Marine Sciences, Center for Wetland Resources, Louisiana State University, Baton Rouge, Louisiana, 70803.

There are a number of hypotheses for the causes of patchiness in marine ecosystems:

(1) physical/biological coupling, *i.e.*, biological patterns resulting from physical oceanographic processes such as advection/turbulent diffusion, vertical mixing, convergences, upwellings, eddies, and local circulation regimes formed by internal waves, Langmuir and Benard cells;

(2) environmental forcing, *e.g.*, gradients or discontinuities in sunlight, turbidity, nutrients, and grazing/competition pressures; and,

(3) behavioral phenomena, *e.g.*, aggregative spawning behavior (especially important in subtropical waters where egg incubation times can be less than 24 h) and planktonic cannibalism or schooling.

The complexity of these patch forcing-functions presents a formidable obstacle to a better understanding of general plankton patch dynamics. In addition, the study of ichthyoplankton patchiness at the microscale or even fine-scale is particularly difficult. Although pelagic marine fishes have high fecundities, the occurrence of their eggs and larvae in the plankton is a relatively rare event, *i.e.*, concentrations are usually low, 0-10 larvae/m^3, as opposed to microzooplankton densities which are often 5-6 orders of magnitude greater (Houde and Lovdal 1985). This is one reason why patchiness in marine invertebrate plankton has been studied more often (see Longhurst 1981 for review), and why the occurrence and morphology of larval fish patches have generally been undocumented (Houde and Lovdal 1985).

The purpose of this paper is to document the fine-scale spatial distribution of red drum (*Sciaenops ocellatus*) larvae off the Louisiana-Mississippi barrier islands, a major spawning area, during their peak abundance in 1986.

MATERIALS AND METHODS

The field effort consisted of six 2-d cruises off the Louisiana-Mississippi barrier islands (Fig. 1) during 5-6 August, 8-9 and 22-24 September, 7-8 and 27-29 October, and 17-19 November 1986. At the start of each cruise, we released 3 windowshade, subsurface current drogues. Drogues consist of a 1.5 × 3 m section of indoor-outdoor carpet suspended 1.7 m below a buoyed radio transmitter, antenna and strobelight. Drogues were tracked visually, by radar, or with a directional radio receiver. Drogue positions were determined every three hours with onboard Loran C.

The sampling design consisted of a 4 × 4 grid of stations occupied on two consecutive nights from sundown to completion of the 16 plankton tows (duration about 5-6 h), and a 3 × 3 grid of stations occupied during daylight, starting 12 h after the initiation of the first nocturnal sampling. The sequence of the station collections within each grid was determined from a random numbers table. Perpendicular inter-station distances were approximately 232 m (1/8 nautical miles), hence, each side of a 4 × 4 grid was roughly 695 m long. The grid had a fixed compass orientation with respect to the 3 drogues, such that the sampling matrix was always kept centered around the drogues' trajectory. This sampling approach maximizes the probability of repeatedly and randomly sampling within the same parcel of water. Surface water temperature and salinity, and water depth were recorded at each plankton station.

Horizontal, 3-min surface tows were taken at a towing speed of approximately 1m/s. All ichthyoplankton collections were made with a 60-cm "bongo type" plankton sampler fitted with 202 μm mesh nets and a flowmeter. Ichthyoplankton samples were field fixed in either 10% buffered formalin (later transferred in the lab to 4% formalin) or 70% ethanol.

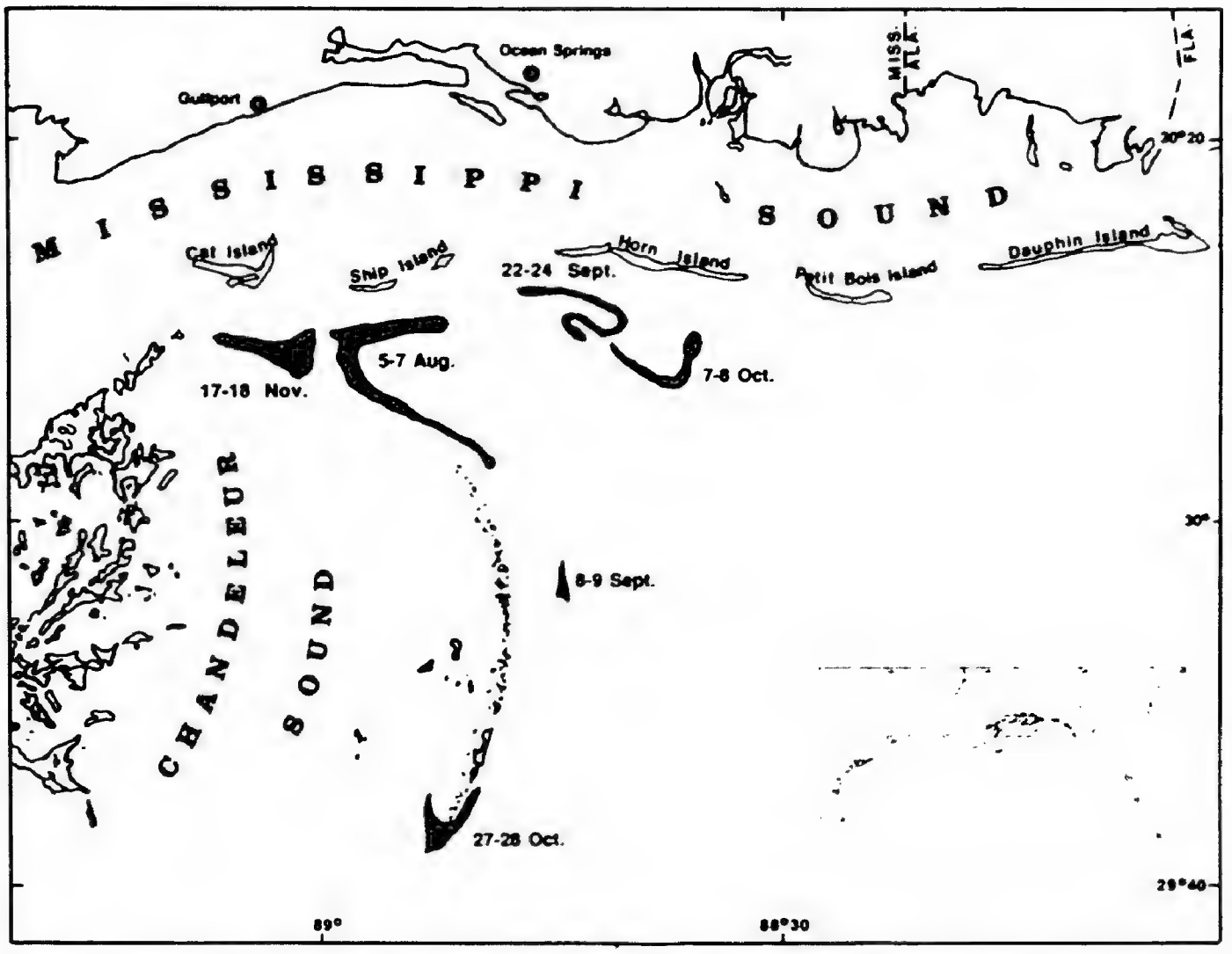

FIG. 1. Location and dates of six two-day ichthyoplankton cruises during 1986. Shaded areas represent trajectories of current drogues and ichthyoplankton sampling areas during each cruise.

A total of 202 tows were taken. Samples were split in half with a Folsom plankton splitter (Van Guelpen, Markle and Duggan 1982) and sorted for all red drum larvae. Two measures of spatial pattern were calculated:
David and Moore's (1954) index of clumping, a density dependent measure given by

$$I = (s^2/x) - 1,$$

where S^2 = sample variance, and x = mean density; and Lloyd's (1967) index of patchiness, given by

$$P = [x + ((s^2/x) - 1)]x^{-1}.$$

Lloyd's index has biological meaning as a measure of the degree of crowding an individual experiences relative to the average crowding expected for a randomly dispersed population, and is independent of density and sampling scale (Pielou 1977, Hewitt 1981, McGurk 1986). Both indices measure the intensity of pattern or the degree of variation in density from station to station, but not the scale or relative spatial extent (Hewitt 1981). Values > 1 for both indices indicate aggregation, patchiness or overdispersion.

RESULTS

During the study (5 August to 19 November 1986), surface temperature and salinity measurements ranged from 18.0 to 29.0°C and 28.5 to 35.0‰. Station

depths sampled ranged from 4 to 15 m. The 22-24 September 1986 cruise was the only sampling period where high densities of larval red drum ($\bar{x}$ = 73.5 larvae/100 m^3) were encountered. Mean density for the 5-6 August cruise was 0.0 larvae/100 m^3, 8-9 September cruise (0.4 larvae/100 m^3), 7-8 October (0.1), 27-29 October (0.1), and 17-19 November 1986 (0.0). Therefore, only the late September cruise data were used in the analysis of spatial variability. During that cruise station depth ranged from 10 to 13 m. Surface temperature and salinity ranged from 28.0 to 29.0°C and 32.0 to 34.0‰, which agrees with optimal rearing conditions for egg incubation and larval development and growth, *i.e.*, temperatures from 22 to 30°C and salinities from 25 to 35‰ (Henderson-Arzapalo 1987, Holt 1987). Only our early November hydrographic data were outside of these optima.

The mean density for the first nocturnal 4 × 4 grid sampling on 22-23 September was 35.7 larvae/100 m^3 (variance = 343.0; range = 3.0-73.0). Mean density the next morning for the 3 × 3 grid was 8.0 (93.2; 0-31.7). For the second nocturnal sampling, mean density was 155.4 (5,240; 15.5-331.6). This density variability was observed over a relatively small sampling area, 0.48 km^2 for the 4 × 4 grids and 0.22 km^2 for the 3 × 3 grid, and with a sampling design which maximized the likelihood of repeatedly and randomly sampling within the same water mass as it was advected by the local current field.

We calculated values for David and Moore's (1954) index of clumping (I) and Lloyd's (1967) index of patchiness (P) using larval densities from each station for the 3 grids. For the first set (4 × 4 station grid) of nocturnal sample densities, the calculated values for I and P were 8.61 and 1.24, respectively. The values for the day samples (3 × 3 grid) were I = 10.59 and P = 2.32, and the second set of nocturnal samples were I = 32.73 and P = 1.21. All values obtained from both indices were > 1 indicating an overdispersed distribution and at least a moderate degree of aggregation or patchiness. The high variability in densities is best seen at the sampling station level (Fig. 2).

DISCUSSION

The distinct 1986 red drum spawning peak which we observed in our plankton collections was further confirmed by gonadal analyses on adult red drum from the same area. September-caught females had the highest percentage of vitellogenic oocytes, and the only occurrences of hydrated eggs were found in late September to early October fish (Fitzhugh, Snider and Thompson 1988). Our plankton tows traversed an average distance of 200 m. The perpendicular and diagonal distances between plankton stations in our sampling grid were approximately 232 m and 328 m, respectively. We observed high variances and patchiness in larval red drum densities at a horizontal scale of 230-700 m over a 30 h period, while attempting to repeatedly sample the same water mass. Few ichthyoplankton studies to date have demonstrated patchiness on smaller or comparable horizontal scales (*e.g.*, 60-400 m, Matsushita, Shimizu and Nose 1982; 10-1000 m, Houde and Lovdal 1985; 300-3000 m, Leis 1982).

NUMBERS OF RED DRUM LARVAE PER 100M3
DURING 22–24 SEPTEMBER 1986

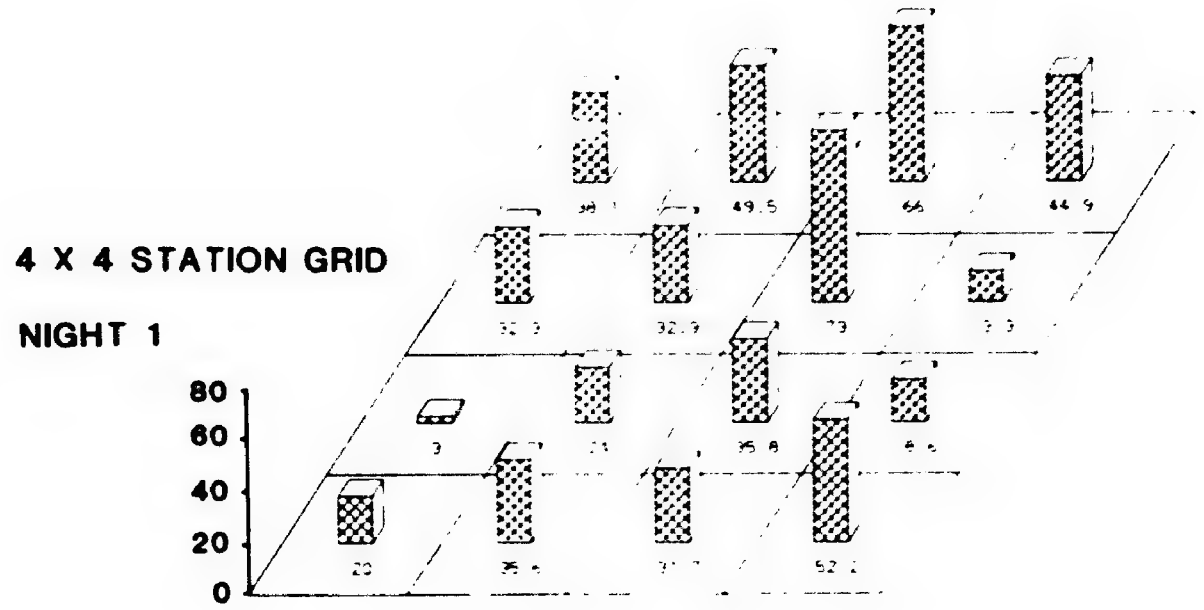

FIG. 2. Spatial distribution of larval red drum densities from a 30-h period (night/day/night) on 22-24 September 1986. Histograms represent numbers of larvae per 100 m^3 from sampling grids of 16, 9 and 16 plankton stations taken around three current drogues. Note change in density scale for each grid.

The high daytime value for Lloyd's index (P), as well as the relatively high daytime value of David and Moore's index (I), may have resulted from density distributional changes induced by vertical migration or daytime net avoidance. The range in nocturnal values (8.61-32.73) for the index of clumping (I) clearly reflects its density dependent nature. However, the nocturnal values for Lloyd's index (P), the most widely used index of spatial patchiness, were virtually identical in spite of the fourfold difference in mean nocturnal densities. In addition, Lloyd's index indicates that the intensity of pattern or the degree of variation in nocturnal red drum densities from station to station did not change significantly over the 30 h observation period.

The observed horizontal patchiness of larval red drum densities probably reflects to some extent, the adults' aggregative spawning behavior. Such nocturnal, synchronous and short-duration spawning behavior (Holt, Holt and Arnold 1985) should initially result in a very dense egg patch whose integrity and morphology may decay over time as a result of dispersal (advection/turbulent diffusion) and predation. Although the integrity and morphology of that initial egg patch may decay, it is not clear how the pattern or patchiness of the larval red drum distribution changes, since some degree of larval patchiness must be maintained by fluctuating grazing pressure, food gradients, and current fields. Since red drum settle out of the plankton before reaching 8 mm standard length (Peters and McMichael 1987), we do not expect their patchiness to increase at a later time because of behavioral factors, as postulated for some pelagic schooling postlarvae (Hewitt 1981).

Time series analysis on density, ontogenetic stage, length frequency, and age/growth are planned to further illuminate patch integrity and evaluate sampling design.

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THE RNA-DNA RATIO: MEASUREMENT OF NUCLEIC ACIDS IN LARVAL *SCIAENOPS OCELLATUS*

Mark E. Westerman and G. Joan Holt

The University of Texas at Austin
Marine Science Institute
Port Aransas, Texas 78373

ABSTRACT

The RNA-DNA ratio has been shown to be correlated with growth in several species of larval fish; however, measurement of nucleic acids in individual larval fishes was limited by the sensitivity of the assays used in these studies. Here we describe a modification of a sensitive enzymatic microassay where the total DNA and RNA of a single 7 day posthatch *Sciaenops ocellatus* was measured. Faster growing larvae had significantly greater RNA-DNA ratios than slower growing cohorts on both days tested. Furthermore, the RNA-DNA ratio was correlated with average growth rate of individuals on days 10 and 18 in larvae of this species. Results of this study are in agreement with earlier work that indicate the RNA-DNA ratio to be indicative of recent or current growth.

INTRODUCTION

Bulow has proposed the ratio of RNA to DNA to be a sensitive indicator of recent growth in fish (Bulow 1970). The ratio has since been used by several investigators as a tool to examine biological, environmental, and xenobiotic factors which affect the growth of fishes (Bulow 1987). Buckley measured the RNA-DNA ratio in 8 species of laboratory reared larval fish and constructed a model based on the RNA-DNA ratio and temperature. The model explains 92% of the variability in protein growth rate (Buckley 1984). However, use of a modification of the Schmidt-Thannhauser method which utilizes UV absorption to quantify the nucleic acids (Munro and Fleck 1966), limited examination of an individual larva to those greater than 0.8 mg dry weight (Buckley 1984). For many species, pooling larvae would result in loss of information on individual nucleic acid metabolism for most of the larval stage. Fluorometric techniques are considerably more sensitive and require less tissue for analysis. Here, we describe a modification of an assay that allows measurement of both RNA and DNA from a single larva (Bentle 1981). Preliminary data on nucleic acid concentrations and the RNA-DNA ratio in 1 through 18 day posthatch larval red drum *Sciaenops ocellatus* is presented.

MATERIALS AND METHODS

Animals

Larvae of the red drum (*Sciaenops ocellatus*) were obtained from several different laboratory spawns and maintained in 200 liter tanks, 1.8 meters in diameter. Larval fish densities were 5 per

liter with 5000 prey/liter. Larvae were fed rotifers for the first 10 days posthatch and then brine shrimp nauplii for the remainder of the rearing period. Salinities varied from 24-32‰ and temperatures from 22-24°C. Two hours prior to assays, larvae were placed in clean filtered seawater to allow for gut clearance. They were then measured and immediately frozen on dry ice until homogenization. Growth rates are expressed as an average increase in length and were calculated assuming a mean hatching length of 2.59 mm. Dry weight of larvae was calculated from the regression,

$$W = 196.044 - 148.940L + 31.180L^2$$

as described by Lee, Holt and Arnold (1984).

Buffer Solutions

All reagents and enzymes were purchased from Sigma Chemical Co. Solutions were prepared in glass distilled, deionized water, with pH adjusted to 7.5 for all buffers. PH was adjusted using 1 M NaOH or 1 M HCl.

Buffer #1
80 mM Tris acetate
4.0 mM $MgCl_2$
3.2 mM $CaCl_2$

Buffer #2
25 mM Tris acetate
1.0 mg ml B.S.A.

Buffer #3
25 mM Tris acetate
5.0 mM $MgCl_2$

Enzyme Solutions

Proteinase K (Type XI, *Tritirachium album*) was dissolved at a concentration of 1.5 mg/ml in Buffer #1. Deoxyribonuclease I (Type II, bovine pancreas) was dissolved to 1.0 mg/ml in Buffer #2. Ribonuclease A (Type III-A, bovine pancreas) was dissolved to 5.0 mg ml in Buffer #2 and heated to 37°C for 15 minutes and then slowly cooled. Enzymes were stored at −20°C in small aliquots.

Nucleic Acid Standards

Highly polymerized DNA (Type I, calf thymus) was dissolved to 0.1 mg ml in Buffer #3. The solution was stirred continuously at 4°C for 24 hours to dissolve DNA completely. RNA (Type III, Bakers yeast) was dissolved to 0.15 mg ml in Buffer #3.

Tissue Preparation

Single larvae were homogenized in chilled 2 ml Wheaton glass homogenization tubes for 1 minute (8 vertical strokes, 400 rpm) in 800 μL of 1 M NaCl. All homogenization procedures were carried out in an ice bath. Homogenates were immediately centrifuged at 3000 × g for 30 minutes at 4°C and duplicate 100 μL aliquots were placed in 12 × 75 borosilicate cuvettes for fluorometric analysis.

Fluorometric analysis

One ml of 5 μg ml ethidium bromide solution, 400 μL of distilled, deionized water and 100 μL Proteinase K were added to the samples. The samples were vortexed and placed in a water bath at 37°C for 45 minutes. Blanks were prepared using 1 M NaCl to replace the sample volume. After incubation, 400 μL of buffer #1 was added and the samples allowed to cool for 5 minutes.

Fluorescence values were then determined on a Turner 111 fluorometer fitted with a multimeter and filters to provide 360 nm excitation and 590 nm emission. Ten μL of the RNase solution was added and the samples replaced in the water bath for 30 minutes. Samples were then removed, allowed to cool for 5 minutes, and fluorescence values recorded. Ten μL of DNase solution was added and samples replaced in the water bath for an additional 30 minutes. Final fluorescence values were then determined.

Calculations

Fluorescence due to RNA was determined by subtracting the second fluorescence value from the first. Fluorescence due to DNA was the third fluorescence value subtracted from the second. These dimensionless fluorescence values were then compared to the standard curves for RNA and DNA and the concentrations predicted by fitting values to the regression equation described by their respective standard curves.

RESULTS AND DISCUSSION

This modification of Bentle's technique is a sensitive and reproducible method for measurement of nucleic acids in larval fish. Accurate measurement of the total RNA and DNA of single, 7 day posthatch larvae was possible. This represents 30-40 μg of dry weight tissue. Pooling 3-10 larvae was necessary for days 1-6 posthatch. Variation in the RNA-DNA ratio between duplicate aliquots averaged 1-3%.

Mean nucleic acid values and their ratio for newly hatched red drum through 18 days posthatch are shown in Figure 1. Total RNA and DNA per

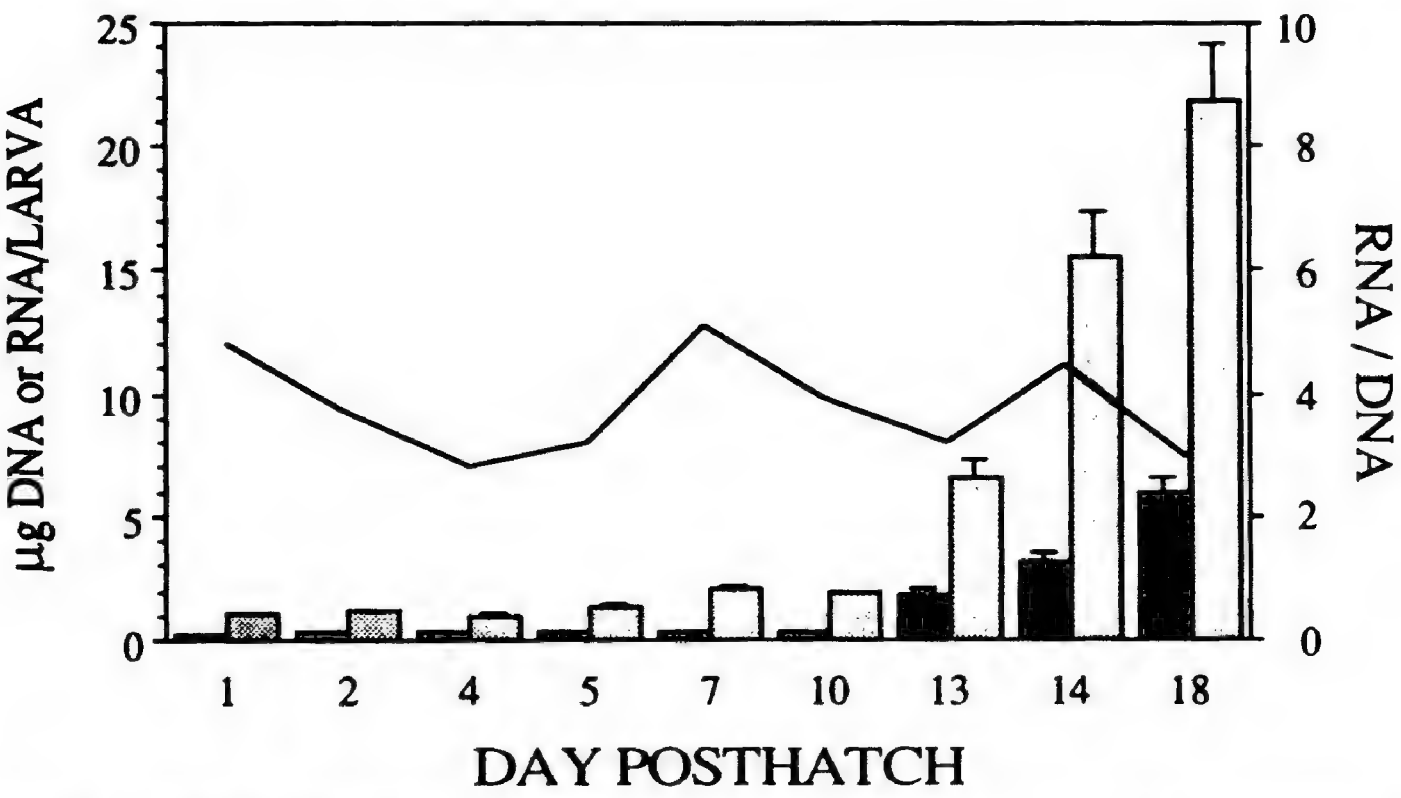

FIG. 1. Mean values for DNA and RNA in micrograms with the RNA - DNA ratio overlaid for individual red drum larvae through 18 days posthatch. Error bars represent one standard error.

larvae increased, though at different rates, with age through 18 days. The rapid increase of total RNA and DNA after 10 days posthatch correlates well

with the rapid increase in dry weight which occurs at this time (Lee *et al.*, 1984). RNA and DNA averaged 8.12 and 1.98% of animals dry weight respectively. This is similar to average values of 1.0 and 5.0% reported by Buckley and Bulow (1987). The higher levels of both nucleic acids in larval red drum may be due to the increased sensitivity of the fluorometric assay (Bentle, Dutta and Metcoff 1981), or to differences in the age and species sampled (Love 1970).

The RNA-DNA ratio decreased from 4.9 in newly hatched larvae to 3.0 at 4 days posthatch (−38.7%). This period is characterized by development driven by maternally derived lipids and yolk proteins (Fig 1). Between 4 and 14 days posthatch, there was a 10 fold increase in amounts of both nucleic acids per larvae and a 60% increase in the RNA-DNA ratio. This indicates rapid cell proliferation accompanied by a high rate of protein synthesis (Bulow 1987). Development of internal organs, jaws, fins, and the onset of feeding, dominate this stage of development. RNA and DNA increased by 36% and 87.5% respectively between 14 and 18 days posthatch. This large increase in DNA relative to RNA suggests a period of hyperplasia, as DNA concentration per unit weight can be considered an indication of cell number (Bulow 1987). In preliminary experiments, groups of 5-10 thirteen and fourteen day posthatch larvae from the same spawn were sampled and assayed for nucleic acids. Large and small size classes were determined visually, with sampling random within size classes. On both days tested, the small and large size class larvae had significantly different mean RNA-DNA ratios (Table 1). Regressions for growth rate of red drum larvae *versus* the

TABLE 1

Age, standard length (mm), quantities of RNA and DNA expressed in micrograms larvae, the RNA-DNA ratio, and results of Student's T-test comparing larvae from the small and large size classes of a single spawn.

	SMALL SIZE CLASS				LARGE SIZE CLASS					
Age	Length	RNA ± SE	DNA ± SE	Ratio	Length	RNA ± SE	DNA ± SE	Ratio	*N*	*P*
13	2.97	2.00 ± 0.06	0.60 ± 0.03	3.5	4.15	8.27 ± 0.65	1.87 ± 0.13	4.4	5	0.001
13	3.16	2.62 ± 0.39	1.05 ± 0.12	2.5	4.45	12.49 ± 0.60	3.38 ± 0.08	3.3	5	0.008
14	3.92	8.24 ± 1.58	1.41 ± 0.10	5.7	5.07	27.11 ± 2.72	3.47 ± 0.149	7.8	5	0.05
14	3.73	5.92 ± 0.61	1.95 ± 0.12	3.0	5.08	23.30 ± 1.81	4.87 ± 0.26	4.8	5	0.0001

SE Standard Error *N* = Number *P* = Probability

RNA-DNA ratio were significant in samples on both 10 and 18 days posthatch (Fig. 2a, b).

These preliminary results indicate that within a single spawn of larval red drum, the fastest growing individuals have significantly higher levels of ribonucleic acids and larger RNA-DNA ratios than their slower growing cohorts. These data are in agreement with previous work by Buckley and support his assertion that the RNA-DNA ratio may be particularly useful in analysis of growth of larval fishes as growth during this period is continuous (Buckley 1984). However, the correlation of RNA-DNA ratio with length of an

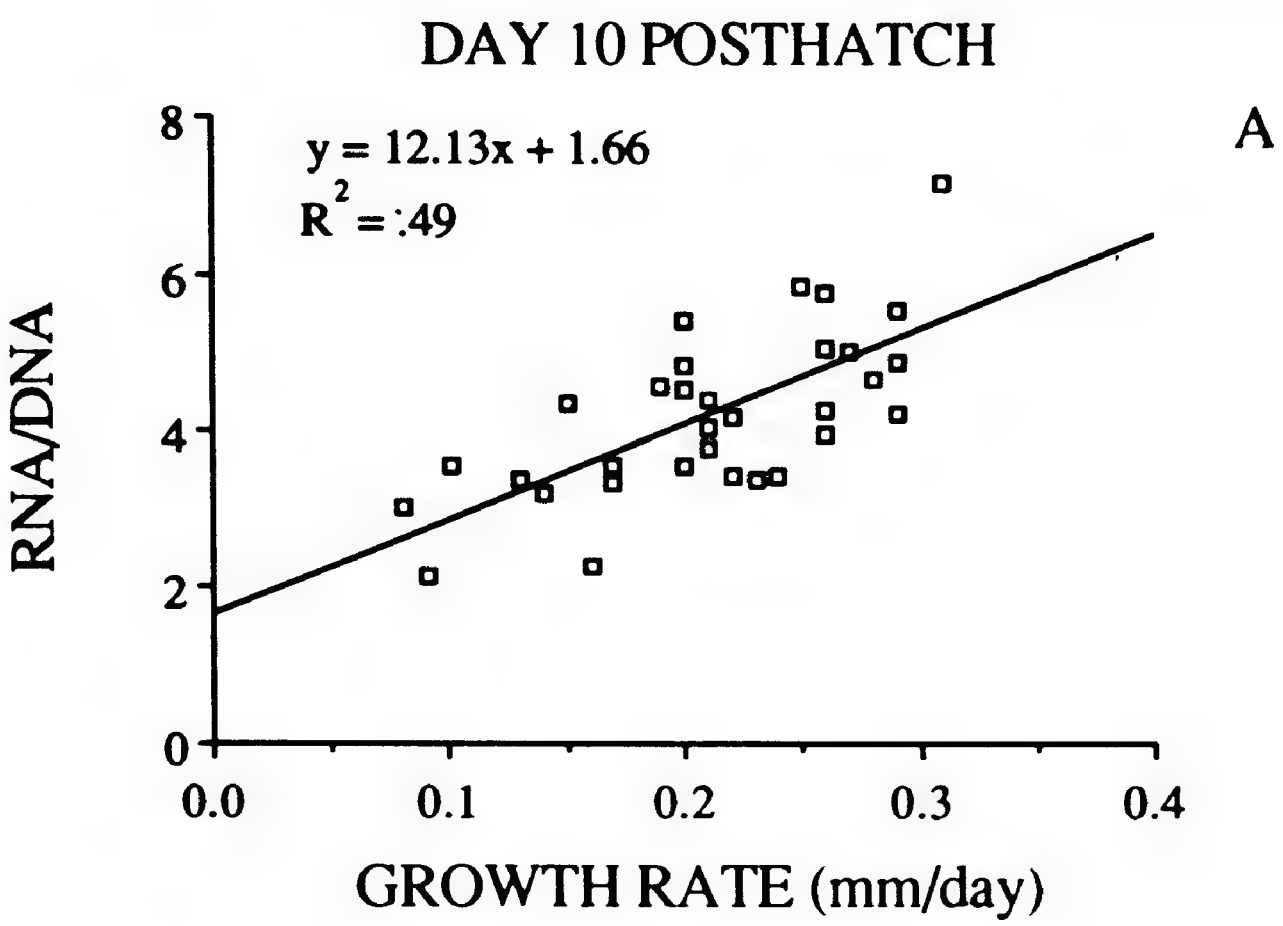

FIG. 2. Regression showing growth rate *vs.* RNA - DNA ratio and R^2 for in.dividual 10 day (A) and 18 day (B) posthatch larvae. Regression was significant to the .0001 level.

individual larva at a given age (Fig. 2a, b) and fluctuations in the RNA-DNA ratio of the populations during the early larval period (Fig. 1) necessitate careful interpretation when using the ratio to assess recent growth of an individual larva or in populations of larvae of unknown age. The RNA-DNA ratio may be particularly useful in analyzing growth of larval *Sciaenops ocellatus* under the controlled conditions typical in an aquaculture setting.

The molecular basis of differences in the RNA-DNA ratio and in growth rate observed among larval fishes of the same spawn are poorly understood. Further studies are necessary to determine how nucleic acid metabolism in larval teleosts is affected by environmental and biological factors that result in increased or decreased growth rates. The amount of DNA per cell is constant among different tissues, while RNA is generally found in higher concentrations in tissues synthesizing protein at high rates (Bulow 1987). Thus, an increase in the RNA-DNA ratio may reflect a general increase in transcription of mRNA coding for metabolic and structural proteins, with subsequent translation occurring on existing ribosomes. However, ribosomal RNA normally represents > 90% of the cellular RNA and therefore changes in the RNA-DNA ratio are most likely driven by either changes in the rate of ribosomal biosynthesis, rate of turnover of ribosomes, or a combination of both mechanisms. Therefore, increased growth rate and RNA-DNA ratio may be the result of an increase in the synthesis of ribosomal RNA and formation of new ribosomes or an increase in the efficiency of ribosomes at initiating protein synthesis coupled with lower turnover of ribosomes in the tissue. An understanding of the class of RNA involved and the nature of these changes will contribute to the interpretation of the relationship between the RNA-DNA ratio and growth in larval fishes.

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FIRST LARVAL REARING TRIALS OF RED DRUM (*SCIAENOPS OCELLATUS*) IN MARTINIQUE (FRENCH WEST INDIES)

P. Soletchnik, E. Thouard, E. Goyard, C. Yvon[1] and P. Baker[1]

INFREMER
Point Fort
97231 Robert, Martinique
French West Indies

ABSTRACT

Two batches of red drum larvae were air shipped from hatcheries in the U.S.A. and stocked in two intensive tank experiments at densities of 7 and 2-3 larvae per liter. Food items include live rotifers, copepods, brine shrimps, squid and commercial fish pellets.

Growth rates were similar in both experiments up to day 20 (12-13 mm) but differences in feeding schedule and an infestation by the dinoflagellate *Amyloodinium* sp. affected the growth from day 20 to 43 in the second trial.

Significant mortalities occurred in the tanks during the first feeding period (days 2 to 7) and again from days 17 to 20 as the result of cannibalism. Survival between day 2 and day 44 was 44% in the first trial. Abnormal inflation of the swim bladder and infection with *Amyloodinium* sp. reduced the survival to 6-19% in the second.

INTRODUCTION

The red drum (*Sciaenops ocellatus* Linnaeus) is a game fish found throughout the Gulf of Mexico. Maintenance of natural stocks is the aim of many larval producers in U.S.A. However, the increased value of the red drum has promoted development of aquaculture of this species. This is the reason research has been directed toward this area for many years.

Maturation and spawning of wild fish under a controlled environment occurred; in Texas (Arnold, Bailey, William, Johnson and Lasswell 1976) and in Florida (Roberts, Harpster and Henderson 1978) the technology is well established. Growth of red drum fingerlings in impoundments was noted by Theiling and Loyacano in 1976 and currently there is interest in improving culture technology using such impoundments. In addition a new field of research is now open on intensive fingerling production in indoor hatcheries.

In Martinique, French West Indies, red drum has been selected as a candidate species for aquaculture development. Because of I.F.R.E.M.E.R.'s experience in intensive systems and the small size of this French island, an intensive approach will be employed to rear this species. This paper presents the results of the first two rearing trials with red drum larvae in Martinique.

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[1] AQUAMAR, Point Fort, 97201 Robert, Martinique, French West Indies

MATERIAL AND METHODS

Larval red drum were imported from the United States; the first batch was obtained from the South Carolina Wildlife and Marine Resource Department (T.I.J. Smith, September 1986) and the second one from the University of Texas (C.R. Arnold, April 1987). Conditions of shipment and environmental parameters recorded before and after the transportation are summarized in Table 1. Newly hatched larvae were stocked in one 400 liter and three 280 liter tanks at two different hatcheries.

An open water system with two sterilization units and a sand filter was set up for the first experiment. Water was recirculated through a sand and biological filter for the second trial.

Environmental parameters during larval rearing were monitored regularly (Table 2). Temper-

TABLE 1

Conditions and environmenal factors involved in transporting of larval red drum

BATCH	1	2
Date of transportation	9/24/86	4/15/87
Number of boxes	5	2
Time of transportation (hours)	39	31
Salinity (initial/Final ppt)	32	31/33
Temperature (initial/final) (°C)	25/25	24/22
Final Ammonia-$NH4^+$ (mg/l)	1,88	0,7-1,0
Density of larvae (N/liter)	1000-1200	1400-2400
Survival rate (%)	5	5-8

TABLE 2

Environmental parameters during larval rearing

	1 (Sept. 1986)	2 (April 1987)
Number of larval rearing tanks	1	3
Volume (liters)	400	280
Initial density (Larvae/liter)	7	2-3
Temperature (°C)	25-28	26-27
Salinity (ppt)	35-37	35-37
Photoperiod (day/night) (hours)	12/12	12/12
Light intensity (lux)	< 1000	300-600
Water change (% hour)		
Larval rearing - day	10-50	0
- night	10-50	0-30
weaning period - day	50-200	8-25
- night	50-200	25-33

ature (25°C - 28°C) and salinity ranges (35‰ - 38‰) were almost the same for the two trials. The rate of water exchange was significantly higher in the first trial compared to the second. Initial stocking densities were 7 and 2-3 larvae per liter, respectively for trial one and two.

Figure 1 shows the feeding scheme of the two rearings. Copepods and two- to six-day old *Artemia salina* were used as feed during the first trial. Commercial dry pellet (60% protein) was used beginning on day 24 for the first trial and day 16 for the second trial. At day 24, the living food (*Artemia salina*) was drastically reduced for the second rearing, while it was fed up to day 32 for the first.

Rearing temperature was just below the optimal at 28-30°C (Lee, Holt and Arnold 1984). Salinities of 35-38‰ were above the natural conditions at 20-35‰ (Holt, Godbout and Arnold 1981, Simmons and Breuer, 1962).

Prey offered for larval rearing were rotifers and brine shrimp, (Lee *et al.* 1984). Feeding schemes differed between the first and second trials with additional copepods and *Artemia* (day 2 to day 6) in the first trial.

RESULTS

Significant mortality occurred during the first day post-shipment for both trials. Up to day 43, survival was higher in the first trial than in the second one (45% *vs.* 81-94%) (Fig. 2). The main cause of mortality for the trial 1 was cannibalism during the weaning period. For the second trial, two additional causes of mortality were:

- problems with swim bladder inflation; between day 2 and day 4, many larvae had hyperinflated swim bladder and died. On day 15-20, non-inflated swim bladders resulted in additional mortalities.
- a dinoflagellate; *Amyloodinium ocellatus* infested the larvae beginning on day 32-34 for 10-15 days.

The growth rate was almost the same for trial 1 and 2 during the two first weeks of rearing as fish reached 11-13 mm length. After this point growth

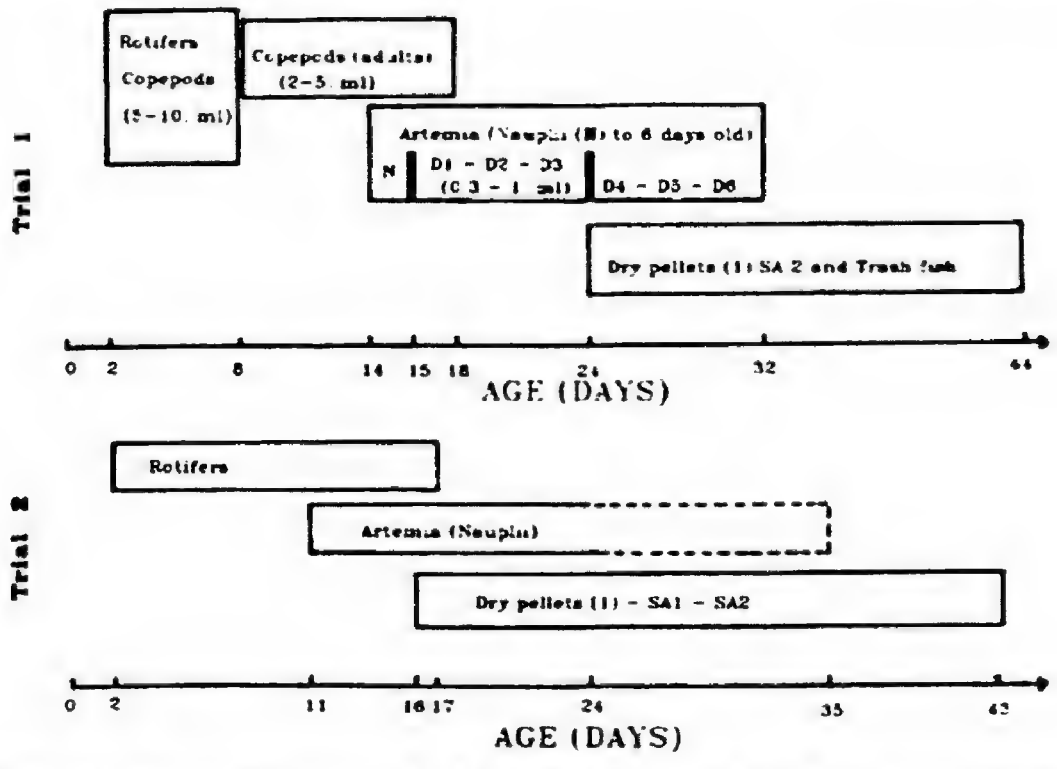

FIG. 1. Feeding schemes for the two larval rearings (trial 1 and trial 2) conducted in hatchery in Martinique (FWI). Dry pellets are commercial trout pellets with range in size from powered (SA0) to 2 mm diameter (SA2).

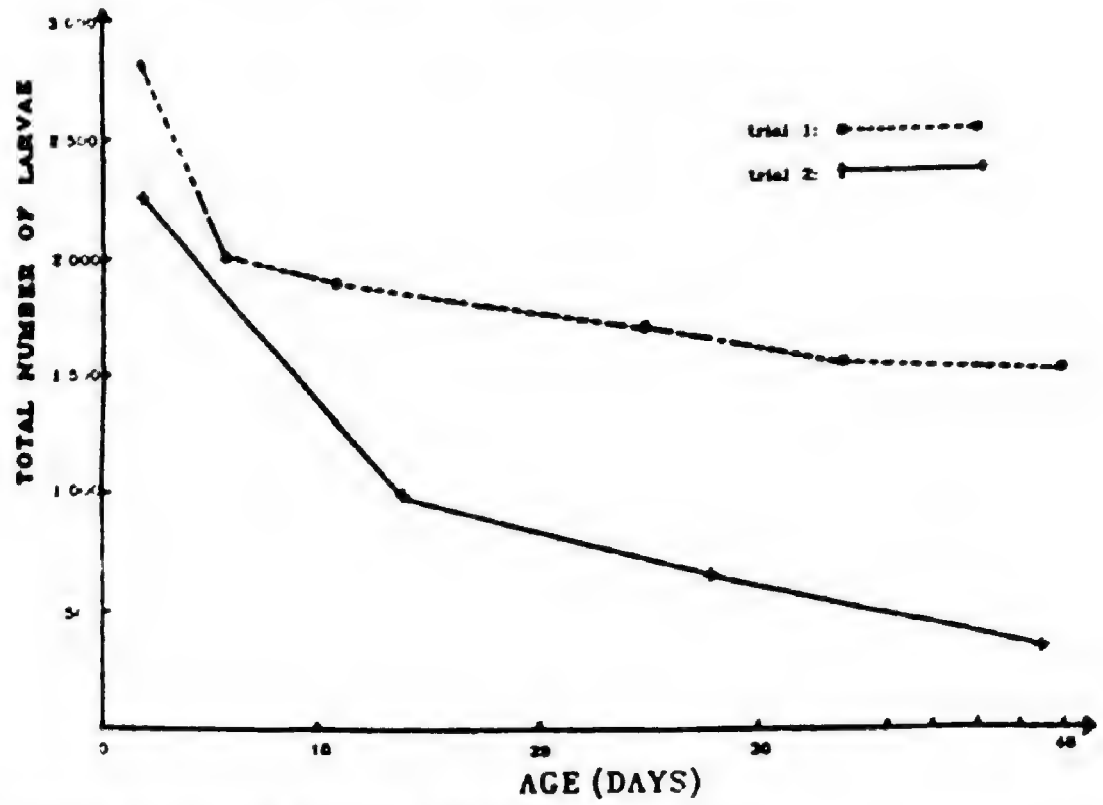

FIG. 2. Survival curves for intensive red drum rearing trials.

rates differed (Fig. 3). At day 43, fingerlings in trial 1 (34 mm) were almost twice those of trial 2 (20 mm).

DISCUSSION

The lengths were not significantly different for the first 20 days. Later the change from living prey to inert food (day 24), the infestation by *Amyloodinium* sp. and the deterioration of water quality, contributed to differences in the two growth rates of the two rearing trials.

The additional food item *Apocyclops distans* (copepod) does not seem necessary. The use of *Artemia salina* (2 to 6 days old) and then the chopped squid mixed with the pellets may have had beneficial effects on growth in trial 1. Results from trial 1 (34 mm standard length in 43 days) are close to the 25-38 mm reached in ponds in 30-40 days (Chamberlain 1984).

In trial 1, cannibalism occurred when size distribution became heterogeneous in the population. Swim bladder problems and *Amyloodinium* sp. infestation, reduced the survival rate to 6-19% in trial 2. Swim bladder problems produced high mortality during the first days (hyperinflation) and then, before metamorphosis, small larvae with non-inflated swim bladders died. Similar results have been reported by Chatain (1986) on the sea bass *Dicentrarchus labrax* which he described as a light dependent problem. The former trouble might have been caused by transportation stress, mechanical shocks, change of temperature or by excessive swallowing of air near the surface, as was noted with *Mugil cephalus* (Nash 1977). The dinoflagellate *Amyloodinium ocellatus* is a well known parasite of the red drum (Paperna

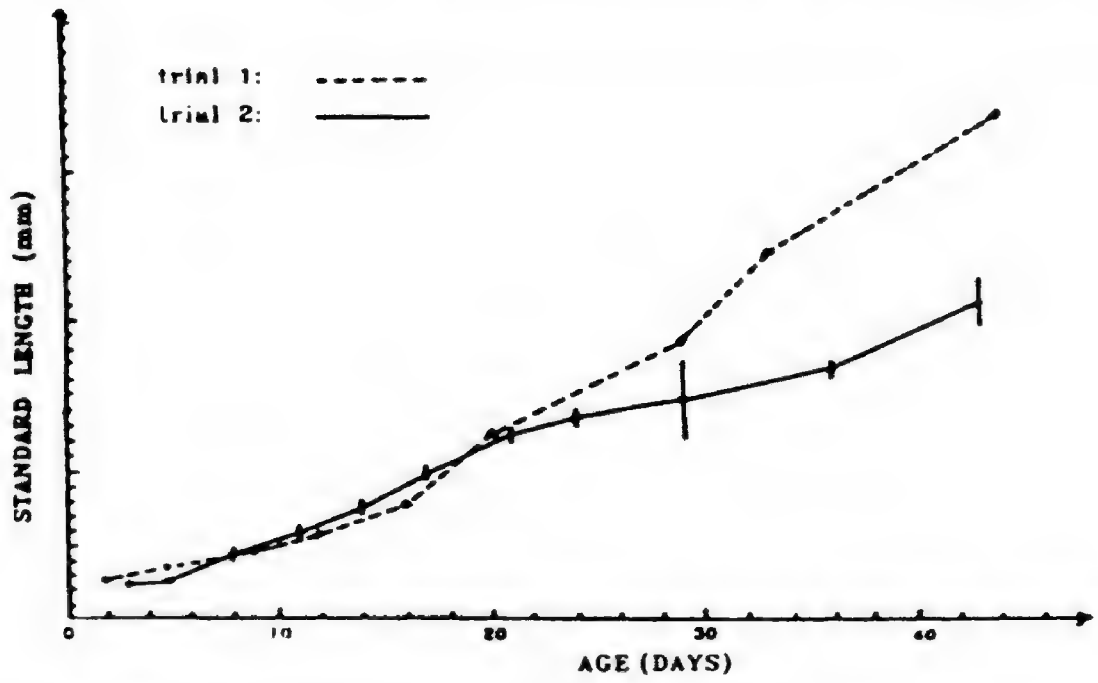

FIG. 3. Growth of red drum during intensive larval rearing trials.

1983). This ectoparasite is also an endemic species in Martinique and has been observed in the rearing of sea bass *Dicentrarchus labrax* in floating cages (D. Gallet, Martinique, 1984, personal communication).

Cannibalism at early development stages constitutes a real problem in the rearing of red drum and especially in intensive culture. It appeared by day 17 when the standard length was 11-13 mm and scales began to appear on the caudal part of the body. Juveniles usually reach fully scaled when they are 25 mm standard length (Holt, personal communication). Differences in the sizes of fish may be a result of the culture system conditions. However, small fishes catch up in size with the larger if they are separated (Holt and Arnold 1985). Holt (personal communication) suggests working with only 1-2 larvae per liter during the third and fourth week of culture and later on, with 1 larvae per two liters.

Larval survival in trial 1 (54%) was similar to the 30-50% obtained in ponds (Chamberlain 1984) and is higher than that typically obtained under intensive laboratory rearing techniques (Chamberlain 1986).

CONCLUSION

Due to limited numbers of larvae, rearing could not be conducted under highly intensive conditions. However, these trials did permit preliminary tests with this new species in the Caribbean Sea. From the first trial, protocols were set up for further investigations. For the next shipment of larvae, a shorter transportation time will be arranged. At that time, research will be conducted to improve the technology for highly intensive larval rearing. Improvements must be made in size selection, feeding scheme and density throughout the rearing period. However it has been demonstrated that red drum can be

raised from eggs to fingerlings in indoor hatcheries under tropical conditions in the Caribbean Sea.

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SECTION IV

Stress Physiology, Disease and Environmental Requirements

P. Thomas, Editor

Contributions in Marine Science. (1988) Supp. to Vol. 30:133–138

HANDLING AND TRANSPORT-INDUCED STRESS IN RED DRUM FINGERLINGS (*SCIAENOPS OCELLATUS*)

J. R. Tomasso[1] and Gary J. Carmichael[2,3]

Department of Aquaculture, Fisheries and Wildlife
Clemson University
Clemson, South Carolina 29634-0362

ABSTRACT

Mortality due to harvesting, handling and transporting red drum fingerlings was evaluated. Groups of fish which were transported about 5 hours generally exhibited less than 1% mortality at the end of the haul. However, cumulative mortality 10 days after the haul ranged from 12% to 51%. Tolerance to net confinement was also evaluated as a relative indicator of the condition of fish before harvesting, after harvesting, and after transporting. Initial results indicate tolerance to net confinement may be of value in determining the condition of red drum fingerlings during aquaculture activities.

INTRODUCTION

The increased demand for red drum (*Sciaenops ocellatus*) as a food fish, coupled with the declining availability of wild fish (Matlock 1982), has produced a keen interest in the commercial farming of red drum. Several states and universities are presently developing culture techniques for red drum, and extension specialists are beginning to develop demonstration projects in some areas.

As in any aquaculture operation, the ability to efficiently and safely transport fish from one point to another will be important to the red drum farming industry. The importance of efficient and safe transport techniques is particularly emphasized when one considers that fingerling production and growout operations will probably be separated in the interest of efficiency. Previous work (Caldwell and Tomasso 1985; Robertson, Thomas, Arnold, and Trant 1987) has shown that red drum are severely stressed when harvested,

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[1] Fisheries Assistance Office, U.S. Fish and Wildlife Service, P.O. Box 39, Pinetop, Arizona 85935

[2] Present address: Aquatic Station, Southwest Texas State University, San Marcos, Texas 78666

[3] Mail Reprint Request to this author.

transported, and stocked. However, little information is available on survival of red drum fingerlings after they are transported. The objective of this study was to determine the effect of stress due to harvesting and transporting on survival of red drum fingerlings for up to 10 days after being transported. The ability of the fingerlings to tolerate a second stressor (Wedemeyer and McLeay 1981) was also investigated to provide further insight into the condition of the fish after handling and transporting.

MATERIALS AND METHODS

Fish (30-35 days old) were obtained from culture ponds operated by the Texas Parks and Wildlife Department. Three of the ponds (two 0.8 hectare ponds and one 1.4 hectare pond) were located on the Dow Chemical Company Facility at Freeport, Texas, and three ponds (0.8 hectares each) were located at the John Wilson Fish Hatchery near Corpus Christi, Texas.

One pond was harvested each day. Prior to harvesting, the pond was slowly lowered over a period of three to four days. Just prior to harvest, the fingerlings moved into the drain kettles and were captured with nylon mesh dip nets. The fish were then transferred into 20-liter buckets containing a known weight of water, weighed, and transferred, by pouring, into the hauling unit.

The hauling unit consisted of a truck-mounted, two-compartment tank constructed of fiberglass covered wood. Each compartment contained approximately 200 liters of water. Adequate dissolved oxygen concentrations were maintained using bottled oxygen and a carbon stone. Salinity and temperature of the hauling water were similar to that of the pond being harvested.

After loading (1.2-2.2 kg of fish per compartment), the fish were transported over a variety of roads for 5 to 8.7 hours. At the end of the haul, one hundred fish were placed into each of four to eight 52-liter fiberglass tanks. These tanks received 1-2 liters per minute of water pumped from Laguna Madre. The fish were fed a moist, sinking pellet (Biodiet [4]) 2 to 4 times per day for 10 days, dead fish were counted and removed daily, and the tanks were cleaned as needed.

The ability of the fingerlings to tolerate a second stressor was determined by placing 50 fish into a small dip net. The net was arranged in a tank so that the fish were crowded and barely submerged. An airstone placed under the net provided aeration. The number of mortalities was recorded after 6 hours. This procedure was repeated for each of three ponds harvested using pre-harvest fish (fish captured from the ponds one day before harvesting), post-harvest fish (fish removed from the pond kettle during harvesting), post-transport fish (fish removed from the hauling tank immediately before stocking into 52-liter recovery tanks), and fish that had been recovering from the transport for 2 and 5 days.

RESULTS AND DISCISSION

Mortality at the end of the transport period was estimated to be less than 1% for fish from 5 of the 6 ponds harvested. Fish from one pond exhibited approximately 10% mortality at the end of the transport period (Table 1). Cumulative mortality for the 10-day post-transport period ranged from 12% to 51%. An analysis of variance indicated that the pond-to-pond mortality varied significantly ($P < .0001$).

During the post-transport period, most of the fish appeared to eat well. No effort was made to quantitate the percentage of fish which were feeding. Most dead fish exhibited shrunken stomachs and were gaunt. Some of the dead fish

[4] Mention of trade names does not imply endorsement by the U. S. Fish and Wildlife Service.

TABLE 1

Summary of conditions and results of red drum harvesting and transport trials. The number of recovery (post-transport) tanks used is given in parentheses under the cumulative mortality.

	Pond 1	Pond 2	Pond 3	Pond 4	Pond 5	Pond 6
Date harvested	7/29/86	7/30/86	7/31/86	5/23/87	5/24 87	5/25 87
Harvest pond salinity (ppt)	35	35	37	35	35	35
Harvest pond temp. (°C)	29	27	26	28	28	28
D.0. at harvest (mg /L)	2.5	1.9	1.5	4.2	3 8	2.9
#fish/kilogram	6,256	4,031	3,115	4,042	2,791	2,974
Hauling tank density (g/L)	10	10	9	13	11	14
Receiving water salinity (ppt)	41	41	41	35	35	35
Receiving water temp (°C)	30	30	30	27	27	27
% dead on arrival[1]	< 1	< 1	10	< 1	< 1	< 1
Cumulative % of mortality after 10 days (mean S.D.[2])	33 ± 3 (8)	12 ± 2 (7)	54 ± 5 (4)	37 ± 9 (4)	40 ± 6 (4)	51 ± 25 (4)

[1] Estimate
[2] Ponds significantly different ($P < 0.0001$)

were also missing all or parts of their caudal fin and caudal peduncle - a condition which was determined to be caused by *Vibrio* sp. in a previous study (Caldwell and Tomasso 1985). Feeding behavior and disease susceptibility may have been different if the fish were released into ponds immediately after transporting. Whether survival in a pond would be higher or lower than in tanks may depend on several factors including food availability, predation, and environmental extremes.

The net confinement studies yielded logical yet variable results (Table 2). Mean mortality of fish from the three ponds after 6 hours of net confinement increased from pre-harvest to post-transport and decreased sharply during the recovery period.

During all of the studies, a great deal of variability in responses from pond to pond was observed. The basis for this variability must be understood if

TABLE 2

Mortality (%) of red drum fingerlings confined in a net for 6 hours. The values represent the mean S.D. of three trials. Each trial used fish from a different pond.

Pre-harvest	20 ± 14
Post-harvest	25 ± 21
Post-transport	57 ± 41
Recovery - 2 day	6 ± 5
Recovery - 5 day	2 ± 2

successful transport techniques are to be developed for red drum. Part of this variability is probably due to the differing temperature and salinity of the culture ponds, which have an effect on both food availability and the bioenergetics of the fingerlings. The condition of the animals at harvest must also be considered. In this study we followed the common practice of counting the number of fish per unit of mass. Perhaps the use of condition factors would provide more insight into the readiness of the animals to deal with transport and stocking stressors.

Further work In the area of red drum transportation techniques will focus on two points. First, a series of transport studies will be carried out using fish maintained under tightly-controlled conditions. Hopefully this will allow the development of a "baseline response" from which various environmental characteristics can be varied to determine optimal transport conditions. Second, the use of net-confinement as a second stressor will be further investigated. If the variability of the response can be reduced, this test might provide insight into the condition of the fish before a pond is drawn down and fish harvested. Ideally, a test such as the net confinement could be correlated with long-term post-transport survival, thus giving the hatchery manager a means of predicting optimal harvesting time.

ACKNOWLEDGMENTS

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THYROID FUNCTION IN RED DRUM (*SCIAENOPS OCELLATUS*)

Duncan S. MacKenzie

Department of Biology
Texas A&M University
College Station, TX 77843

ABSTRACT

Thyroid gross morphology, circulating thyroid hormone levels, and free thyroid hormone indexes are similar in red drum to those found in other teleost species. Differences observed in thyroid hormone levels in animals sampled after growth at different salinities indicate that nutritional or environmental conditions may alter thyroid function in this species. Red drum may potentially serve as an excellent model species for the examination of thyroid function in a cultured euryhaline teleost.

INTRODUCTION

In spite of extensive evidence for a probable role of thyroid hormones in physiological processes of interest to aquaculture (reviewed in Eales 1979; Higgs, Fagerlund, Eales and McBride 1982; Leatherland 1982), studies of thyroid function in teleost fishes are limited to a relatively small number of genera. The present study was undertaken to provide background information on thyroid structure and function in a teleost species of economic and recreational interest, the red drum (*Sciaenops ocellatus*). Although several state or privately owned red drum hatcheries and grow out facilities are currently operating in Texas, virtually nothing is known about the endogenous physiological regulation of growth, metabolism and development in this species, and little information is available on the physiological responses of red drum to environmental manipulation. As thyroid hormones have been implicated in regulating metabolic activity and mediating physiological responses to stress and to varying salinities in euryhaline teleosts (Eales 1979, Leatherland 1982), information on thyroid function in this species may potentially provide a tool for evaluating the effects of imposed culture conditions on metabolic activity. In addition, red drum may provide us with a new euryhaline teleost model system in which thyroid phenomena can be examined in detail, yielding new information basic to the understanding of the mechanisms of salinity adaptation and the role of the thyroid in euryhaline teleosts.

MATERIALS AND METHODS

Fish

Red drum fingerlings (approximately 0.1 g body weight) were obtained from the John Wilson Fish Hatchery, Corpus Christi, Texas, through an agreement with the Texas Department of Parks

and Wildlife. Fish were transported to College Station and maintained at 25‰ salinity, 28°C, 16L:8D photoperiod in a 3000 gallon recirculating artificial seawater (Super Salt, Fritz Chemical Co., Mesquite, Texas) system at the Texas A&M Research and Extension Center (Annex) in Bryan, Texas. Fish were fed 4 times daily with commercial salmon starter diet (Zeigler Bros. Inc., Gardners, Pennsylvania) at a ration of approximately 2.5% body weight per day. Samples of blood and tissue were also obtained from John Wilson Hatchery fish which had been maintained for 9 months in fresh water ponds at the Aquaculture Research Center (ARC) of the Texas A&M Department of Wildlife and Fisheries Sciences. In addition to forage feeding, pond fish were fed twice daily to satiation with commercial trout chow (Zeigler). For fish held in recirculating systems, all blood and tissue samples were taken within 5 minutes of netting. For ARC pond fish, animals were seined from the pond at 0800 hrs and transferred to 1200 L flow-through tanks, then sampled after 4 hours and again after 24 hours.

Sampling

Blood was sampled from the caudal vasculature of restrained, unanesthetized fish into 5 ml syringes containing ammonium heparin; plasma was stored at −80°C until assayed. Heads from five animals at each salinity were fixed in Carnoy's fixative for examination of thyroid follicle distribution using a specific thyroid follicle staining technique (Rosenkilde and Wingstrand 1961).

Thyroid Hormone Binding

Plasma samples from ten 400 g, 25‰ fish (mean weight ± s.e. = 317 ± 24.9) were pooled for analysis of thyroid hormone plasma binding. Free thyroid hormone levels were assayed in this

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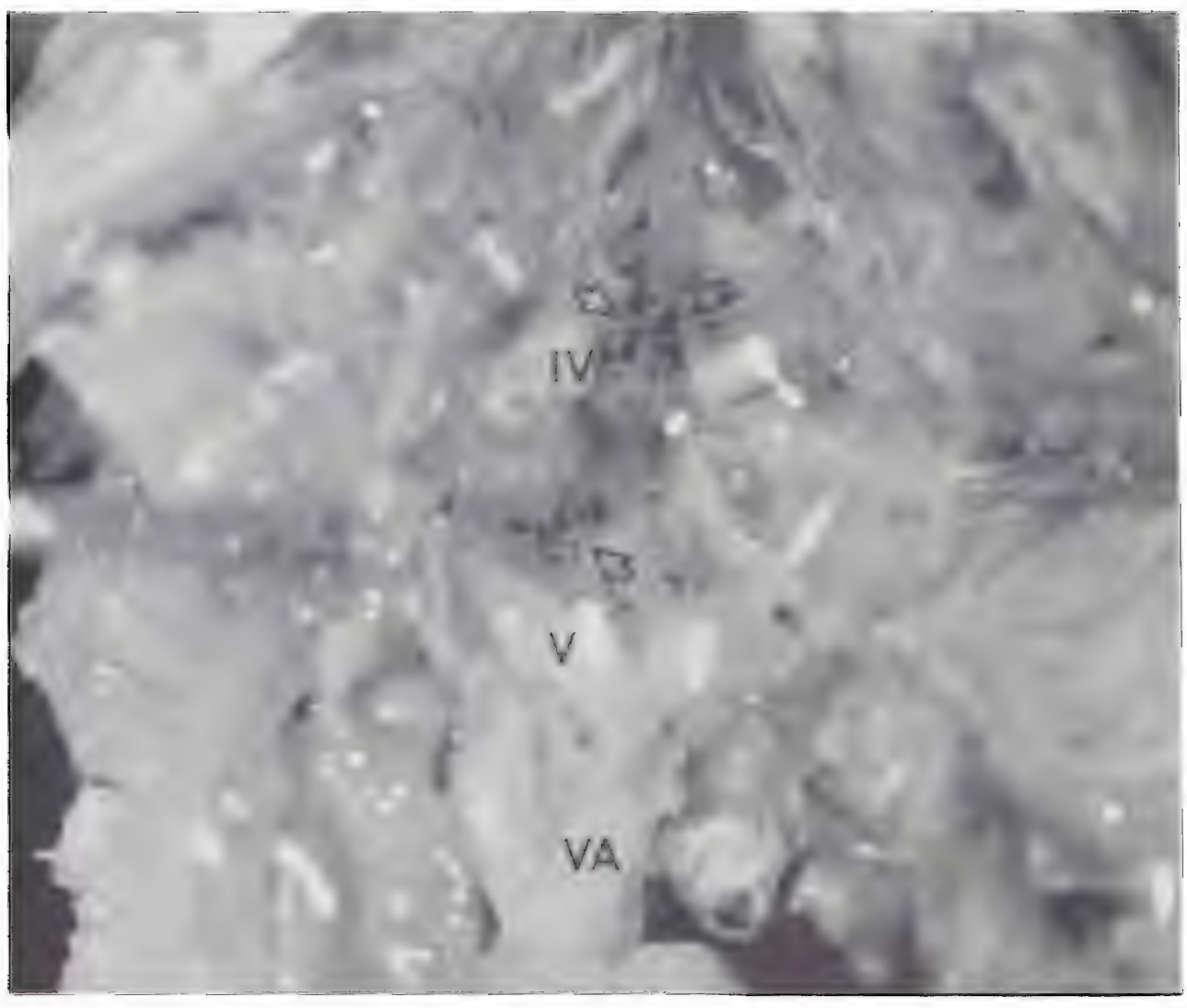


FIG. 1. View looking down into the lower jaw of a juvenile (100g) red drum showing darkly-staining thyroid tissue. Anterior of head is up. The ventral aorta has been removed anterior to the

pool by the Sephadex-binding technique of Eales and Shostak (1985), and molecular weights of triiodothyronine binding proteins were estimated by Sepharose column chromatography using the technique of Mitchell and Stiles (1985).

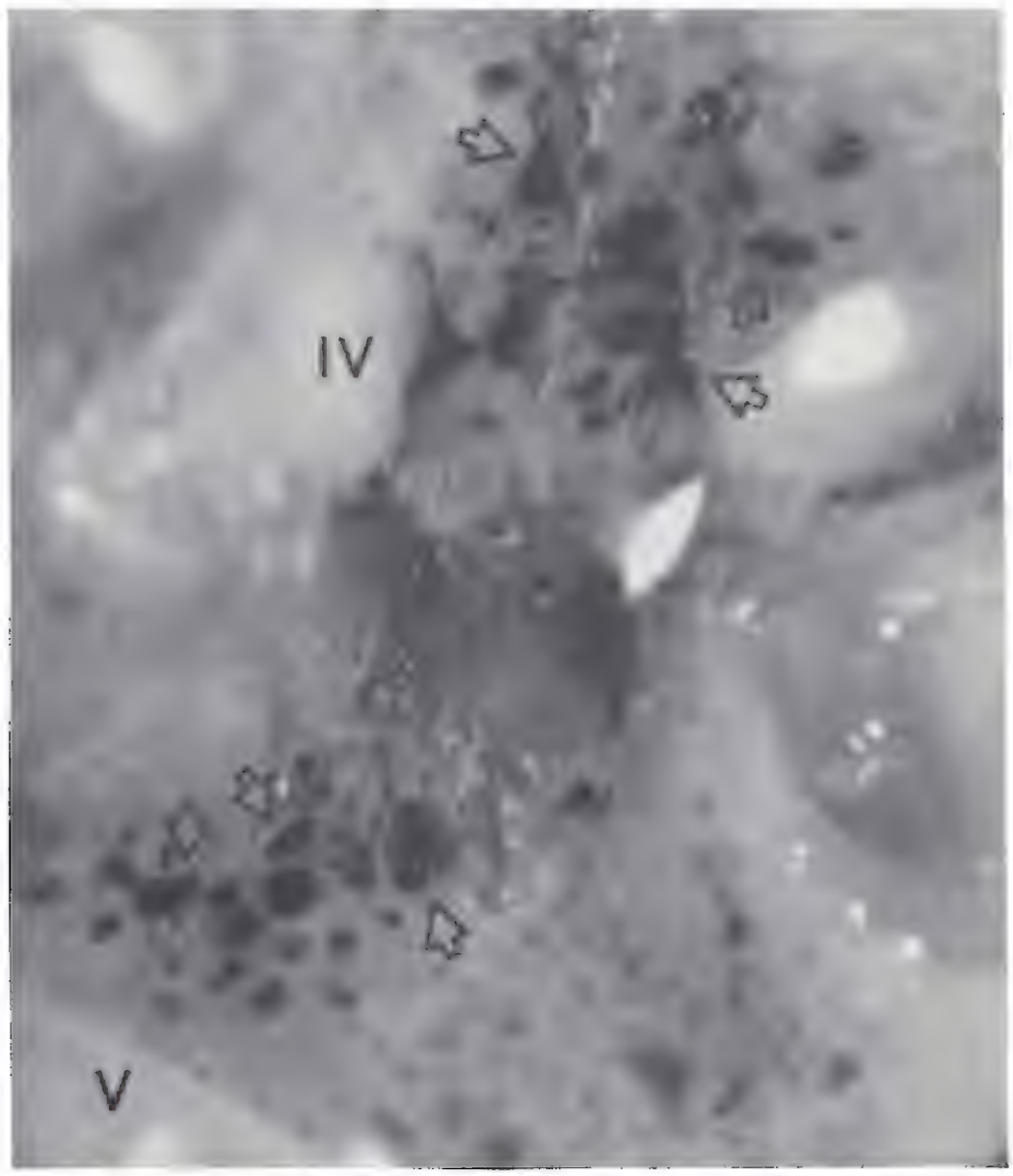
Blood Analysis

Blood levels of thyroid hormones (triiodothyronine, T_3, and thyroxine, T_4) were measured in unextracted plasma using specific radioimmunoassays (MacKenzie, Sokolowska, Peter and Breton 1987). Plasma osmolality was measured with a Wescor model 5100 vapor pressure osmometer. Tests for significance were performed on paired samples using a Mann-Whitney U test, $\alpha = 0.05$.

RESULTS AND DISCUSSION

In animals acclimated to fresh water, the specific thyroid follicle staining technique revealed discrete areas of thyroid tissue scattered along the ventral aorta between the third and fifth aortic arches (Fig. 1). The thyroid gland in

1B



fifth aortic arches (V); the fourth aortic arches (IV) have been cut. Dispersed follicles are visible in the area ventral to the ventral aorta. (a) 20X magnification showing general distribution of thyroid tissue (arrows). (b) 50X magnification showing individual follicles (arrows).

red drum thus appears to be diffuse, as it is in the majority of teleost species (Eales 1979); no discrete, encapsulated thyroid gland, as is found in a small number of teleosts (Grau, Helms, Shimoda, Ford, Legrand and Yamauchi 1986), was evident. Attempts to use the specific thyroid follicle staining technique to locate thyroid tissue in fish taken from high salinity (25-35‰) systems were unsuccessful, as the entire tissue preparation stained very darkly with the alcoholic silver reagent, making it difficult to distinguish thyroid tissue from nonspecific background. This problem may have been due to a relatively high iodine level (basis for the staining reaction) in the tissue of saltwater acclimated fish.

Percent free hormone index (an indication of the availability of metabolically active hormone in the blood; Eales 1985) was determined by examining the ability of serial dilutions of red drum plasma to elute bound T_3 and T_4 tracer from Sephadex minicolumns, using human plasma as a reference standard. Binding of T_3 to red drum plasma was consistently higher than that of T_4 at all dilutions, although lower than binding of either hormone to human plasma. Greater T_3 than T_4 binding, also observed in salmonids (Higgs *et al.* 1982, Eales and Shostak 1985) and ictalurids (MacKenzie *etal.* 1987), may reflect a higher affinity for T_3 by plasma binding proteins, as has been found in salmonids (Eales 1987). Red drum also resemble these other fish in showing higher free hormone indexes than mammals; in the present study, the calculated free hormone index (mean of duplicate determinations) at a dilution of 1:20 was 2.0 for T_3 and 4.2 for T_4 in red drum versus 0.4 for T_3 and 0.1 for T_4 in human plasma. This substantial difference in free thyroid hormone indexes between humans and poikilothermic vertebrates is most likely due to the lack of a high affinity thyroxine binding globulin in poikilotherms (Larsson, Pettersson and Carlström 1985).

To estimate the molecular weights of T_3 binding proteins in red drum, the plasma pool was incubated with radioiodinated T_3 and subjected to gel filtration chromatography (Fig. 2). Four major peaks were observed by optical density measurement at 280 nm, at approximate molecular weights (based on prior column calibration with known molecular weight standards) of 2.5 million, 450,000, 30,000, and 4,000 daltons (d). Radioiodinated T_3 binding was bound exclusively to proteins in the major 30,000 d peak, with primary binding in an area of 25,000 d and a secondary shoulder in the area of 45,000 d. Thyroid hormone binding proteins have not been identified with certainty in teleost fishes, although binding to low molecular weight proteins has been demonstrated in salmon (Larsson *et al.* 1985). Further purification and characterization of proteins in the primary peak are necessary to determine whether this peak contains albumin, proposed to be a major thyroid hormone binding protein in fish (Tanabe, Ishii and Tamaki 1969), or a binding prealbumin resembling mammalian prealbumin in the 45-60,000 d range (Robbins, Cheng, Gershengorn, Glinoer, Cahnmann and Edelnoch 1978).

Serial dilutions of red drum plasma ran parallel to serial dilutions of T_3 and T_4 standards in the radioimmunoassay. Basal levels of T_3 were very similar in animals maintained for long periods at either high or low salinity (Table 1),

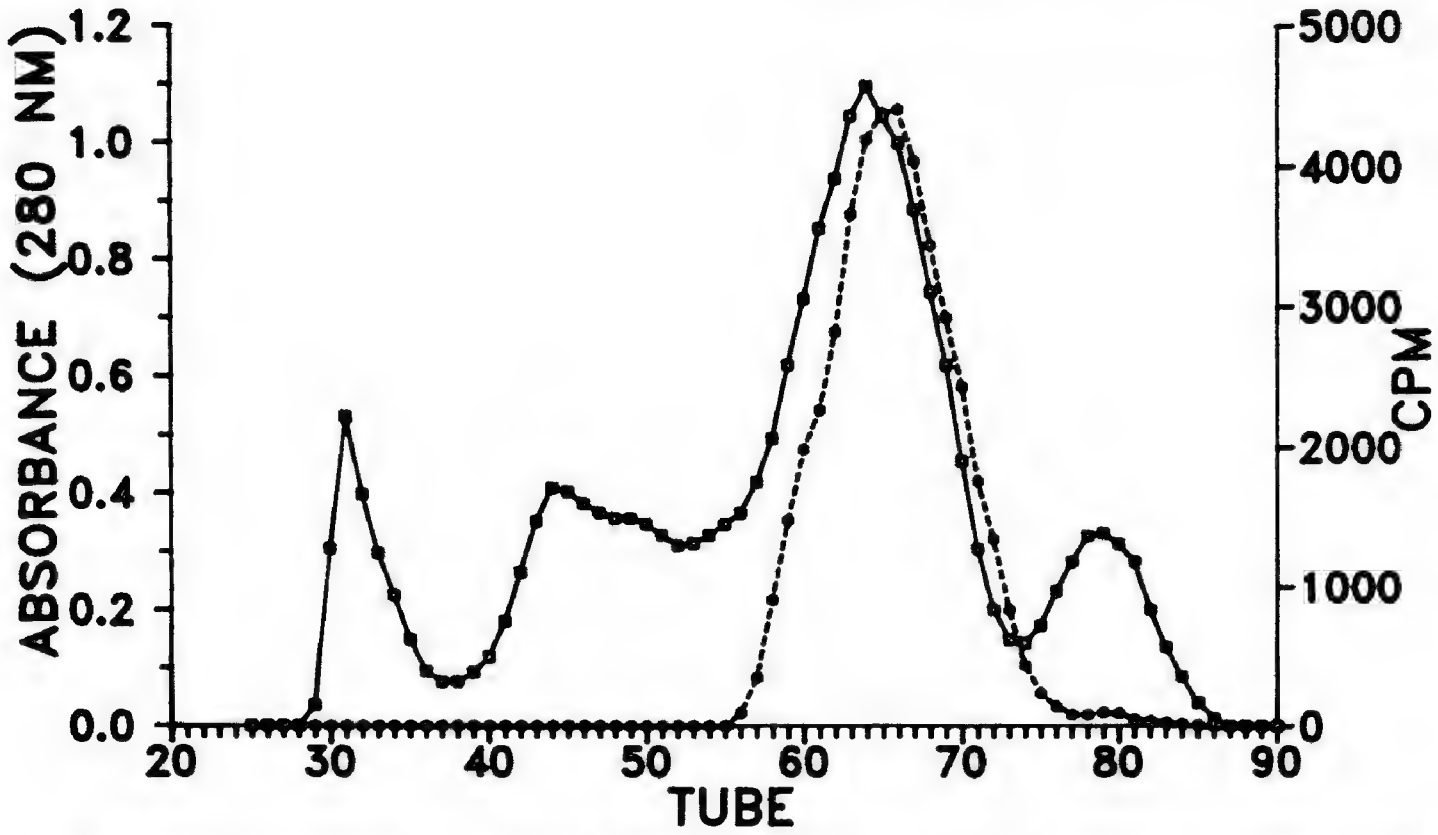


FIG. 2. Elution profile of 1.75 ml pooled red drum plasma applied to a 90 X 1.5 cm Sepharose 6B column at 4° and eluted in Tris-NaCl buffer at 10 mls/hr. Absorbance at 280 nm (solid line) and cpm of bound radioiodinated T_3 (dashed line) were determined in successive 2 ml fractions.

TABLE 1

Thyroid hormone levels in red drum from differing culture conditions and locations.

LOCATION	N	TEMP (°C)	SALINITY (ppt)	WEIGHT (g)	PLASMA OMOLALITY (mosm/kg)	THYROID HORMONES (ng/ml) T_3	T_4
ANNEX	15	26	25	58 3 (2 2)	340 (12.4)	4 1 (0 5)	0 5 (0 2)
ANNEX	9	26	25	317 (25)	337 (9.8)	4 9 (1 1)	0 5 (0 1)
ARC. 3	10	28	1	473 (16)	308 (10.7)	5 6 (0 6)	6 3*(1 2)
ARC. 24	7	28	1	323 (43)	281 (11.9)	4 7 (1 0)	6 9*(2 5)

All values are means ± s.e.
* denotes values significantly different from either Annex group

whereas T_4 levels differed significantly between fresh and saltwater acclimated fish (Table 1). It is possible that this difference was associated with different feeding regimes between the two groups of fish (Higgs *et al.* 1982) or the prolonged stress associated with seining and holding the pond animals, as stress-induced alterations in thyroid hormone levels have been noted in other teleosts (Brown, Fedoruk and Eales 1978). Future experiments will be directed toward examining the effects of stress and salinity transfer on juvenile fish in an attempt to elucidate the physiological and environmental controls regulating circulating thyroid hormone levels.

Thyroid function in red drum thus appears initially to be very similar to that of other teleost species described in terms of thyroid morphology, hormone production, and circulating levels. Because of this, and because red drum are now being grown extensively in controlled culture systems at differing salinities, temperatures, and rations, this animal presents an excellent opportunity for a comprehensive investigation of the relationship between thyroid function (including both total and free thyroid hormone levels as well as plasma protein binding) and environmental conditions in a euryhaline teleost species.

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TEMPERATURE SENSITIVITY OF LACTATE DEHYDROGENASE (A_4-LDH) FROM WHITE SKELETAL MUSCLE OF SCIAENID FISHES

Zulema L. Coppes and George N. Somero

Marine Biology Research Division
Scripps Institution of Oceanography
University of California, San Diego
La Jolla, CA 92093-0202

ABSTRACT

Lactate dehydrogenases (A_4-LDH) from five sciaenid species were purified by oxamate affinity chromatography. The temperature sensitivities of the LDHs were measured by determining the effects of temperature on the Michaelis-Menten constant (K_m) of pyruvate and on the maximal velocity (V_{max}) of the reaction. Although increases in temperature invariably increased the K_m of pyruvate for each LDH, the K_m was highly conserved at the physiological temperatures of the different species. For the red drum (*Sciaenops ocellatus*), the K_m of pyruvate was less perturbed by temperature than for the other species, and the effect of temperature on the maximal velocity of the reaction, as manifested in the Arrhenius activation energy (Ea), was also less for this species. The importance of conserving K_m in the face of variations in environmental and body temperatures is discussed.

INTRODUCTION

Fishes of the family Sciaenidae (Perciformes) are found in environments having widely different thermal regimes. Some species encounter wide ranges of habitat temperature, whereas other species live in more thermally stable waters. The fishes of this family provide, therefore, an excellent study system for examining adaptation to temperature. We compared the thermal sensitivity of the skeletal muscle isozyme form (designated the A_4 or M_4 isozyme) of the glycolytic enzyme lactate dehydrogenase purified from five sciaenid species that occur in different thermal conditions. In broad comparisons of fishes, reptiles, and mammals having different body temperatures, A_4-LDH has been shown to reflect the adaptation temperature of a species (Somero 1986). By extending these studies to a group of confamilial species we hoped to provide a more "fine-scale" image of enzymatic adaptation to temperature. We particularly wished to investigate the temperature sensitivities of LDH from highly eurythermal fishes to see if these enzymes are less affected by variations in temperature than the homologous enzymes from stenothermal species.

MATERIALS AND METHODS

Sciaenops ocellatus, *Leiostomus xanthurus*, and *Cynoscion nebulosus* were supplied by Dr Joan Holt (University of Texas Marine Science Institute, Port Aransas, Texas). Fish were shipped on solid carbon dioxide and stored at −80° until used. The species from the southwest Atlantic, *Cynoscion striatus* was transported on solid carbon dioxide from Montevideo, Uruguay, and the northwest species, *Atractoscion nobilis*, was caught offshore of San Diego.

The A_4 isozyme of lactate dehydrogenase was purified from white locomotory muscle by affinity chromatography on oxamate-agarose, as described by Yancey and Somero (1978), except that 1 mM NADH rather than 200 μM NADH was required to ensure binding of the LDH from one species (*Sciaenops ocellatus*) to the affinity resin. The basis for this difference in affinity to oxamate is not known.

LDH preparations were examined by starch gel electrophoresis to test for purity of the A_4 isozyme following the method of Shaklee, Kepes and Whitt (1973). Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) was used to examine the homogeneity of the proteins

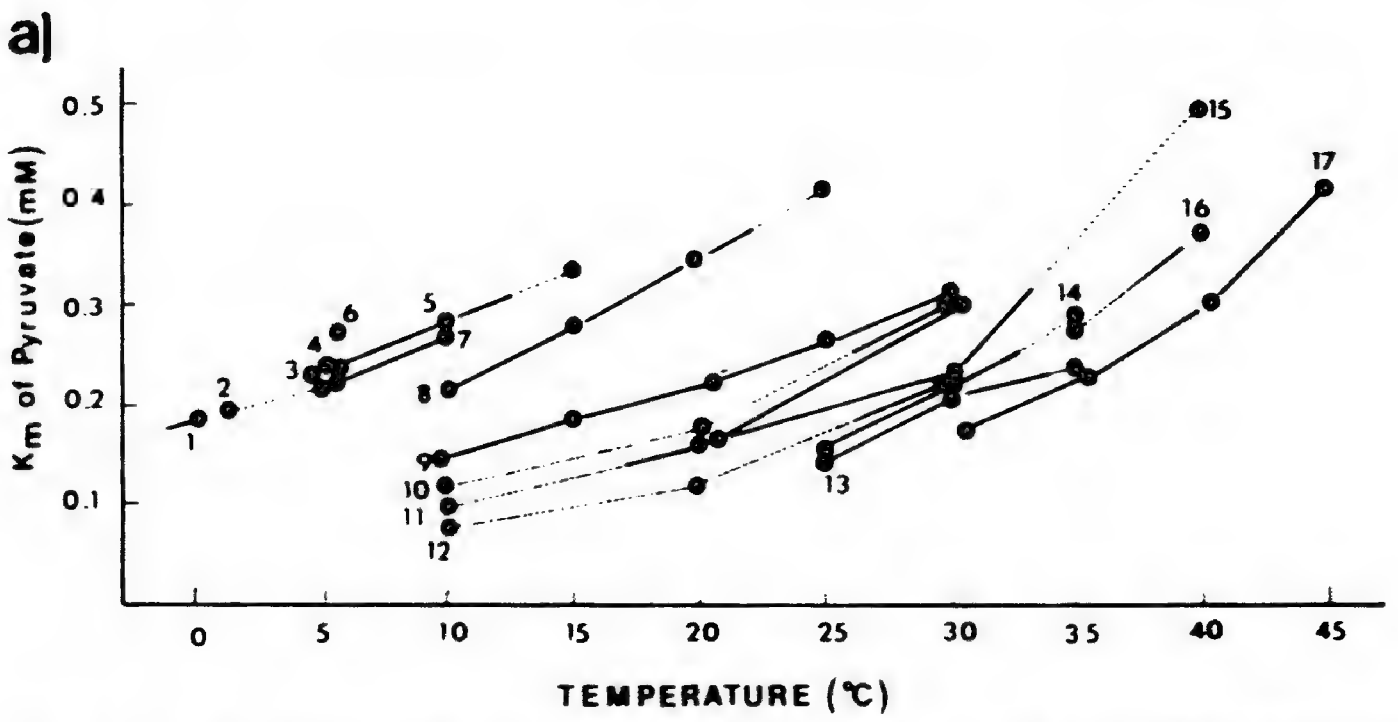


FIG. 1. The effect of temperature on the K_m of pyruvate for A_4-LDHs of vertebrates adapted to different temperatures. The solid lines in each individual plot indicate the physiological temperatures for the species. Upper frame A: data from previous studies (Somero 1986).

1. *Pagothenia borchgrevinki* (Antarctic notothenid fish),
2. *Halosauropsis macrochir* (deep-sea halosaur fish),
3. *Coryphaenoides acrolepis* (deep-sea macrourid fish),
4. *Sebastolobus altivelis* (deep-sea scorpaenid fish),
5. *Sebastolobus alascanus* (scorpaenid fish),
6. *Antimora rostrata* (deep-sea morid fish),
7. *Hippoglossus stenolepis* (Pacific halibut),
8. *Thunnus thynnus* (blue fin tuna),
9. *Gillichthys mirabilis* (longjaw mudsucker fish),
10. *Abudefduf taurus* (damselfish),
11. *Thalassoma lucasanum* (wrasse),
12. *Epinelphelus cruentatus* (grouper),
13. *Potamotrygon* sp. (freshwater Amazon ray),
14. *Hypostomus plecostomus* (Amazon armored catfish),
15. *Gerrhonotus multicarinatus* (southern alligator lizard),
16. *Oryctolagus caniculus* (New Zealand white rabbit), and
17. *Dipsosaurus dorsalis* (desert iguana).

of the purified enzyme. Each preparation contained only the A_4 isozyme of LDH and only a single band of protein was evident on SDS-PAGE.

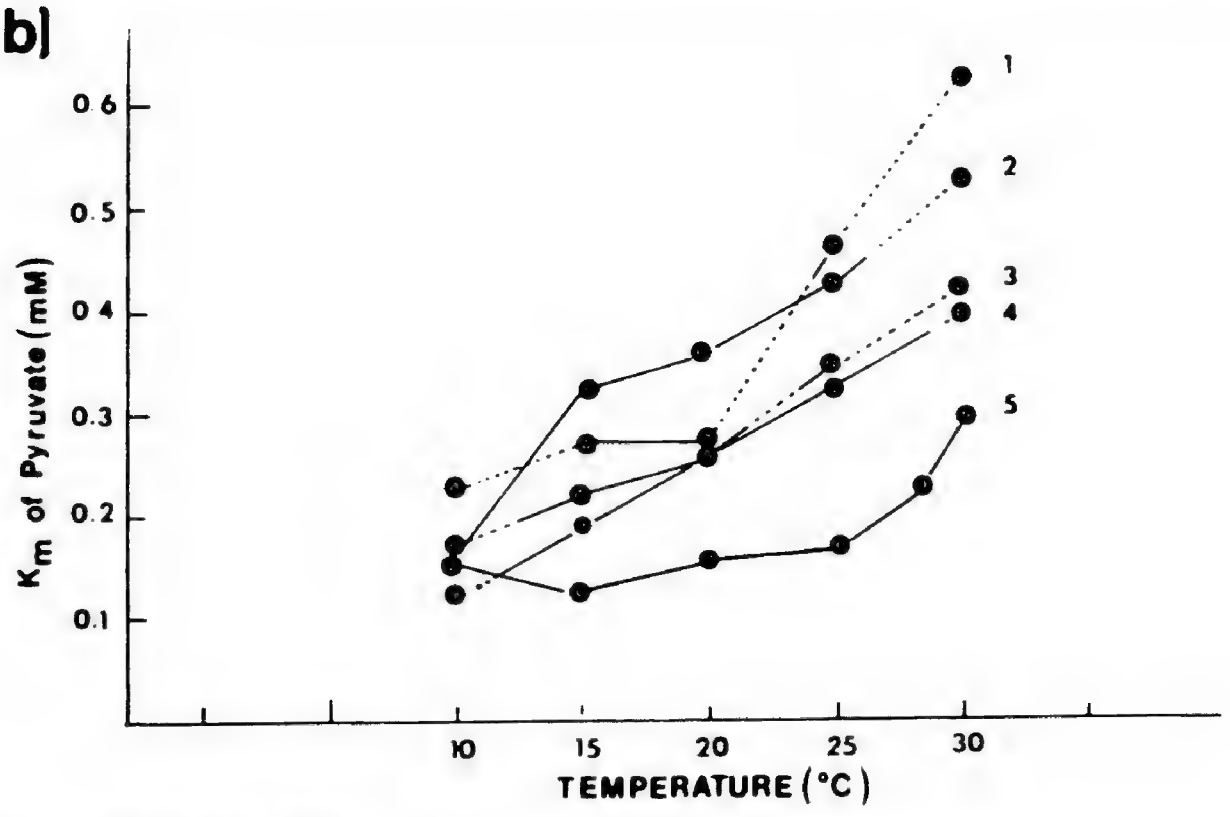
The purified LDHs were stored refrigerated as ammonium sulfate precipitates in the presence of 2-mercaptoethanol. The precipitated enzymes were resuspended in buffer and dialyzed exhaustively for use in the kinetic studies.

Measurements of LDH activity and computation of the K_m of pyruvate followed the methods of Yancey and Somero (1978). An 80 mM imidazole-chloride buffer was used to simulate the physiological pH at each measurement temperature (*cf.* Yancey and Somero 1978).

RESULTS

The K_m of pyruvate values for the LDHs of the five sciaenid species are shown in Fig. 1-b along with published values for LDHs (all A_4 isozymes) from a wide range of other vertebrates (Fig. 1-a). The dark lines connecting the K_m values indicate the temperature ranges encountered by each species. Over the physiological temperature ranges of the sciaenid species, the K_m of pyruvate is conserved within a relatively narrow range, approximately 0.15 to 0.4 mM pyruvate. This is also the range within which the K_m of pyruvate is conserved in other vertebrates having average cell temperatures ranging from near −2°C to over 40°C (Fig. 1-a).

The degree of variation in the K_m of pyruvate with measurement temperature differed among the five sciaenid fishes. Notably, the K_m of pyruvate for LDH of *S. ocellatus* was much less sensitive to temperature than the K_m values



Frame B. Five sciaenid fishes.
1. *Cynoscion striatus,*
2. *Leiostomus xanthurus,*
3. *Atractoscion nobilis,*
4. *Cynoscion nebulosus,* and
5. *Sciaenops ocellatus.*

for the other species' LDHs. This species' LDH also had the lowest Arrhenius activation energy of all LDHs examined in this study (Table 1), a further indication of the relative temperature-insensitivity of this species' LDH.

TABLE 1

Arrhenius activation energies (Ea) for lactate dehydrogenase reactions of fishes of the family Sciaenidae.

Species	Habitat Temperatures (°C)	Ea (cal/mol)
Cynoscion striatus	14 - 18	11,487
Atractoscion nobilis	12 - 20	10,598
Cynoscion nebulosus	10 - 30	12,081
Leiostomus xanthurus	10 - 20	11,509
Sciaenops ocellatus	5 - 30	9,727

DISCUSSION

The A_4 isozyme of LDH functions as the terminal enzyme in anaerobic glycolysis, regenerating NAD in the presence of limiting oxygen supplies (Markert and Ursprung 1962). To function effectively in this role, the enzyme's ability to bind pyruvate must be great enough to ensure a high rate of catalysis, yet binding must not be so strong that the enzyme's active site is saturated with substrate. In the latter circumstance, the enzyme is unable to increase its rate of activity as pyruvate levels rise, as they would, for example, during intense swimming. K_m of pyruvate values thus are held within the range of physiological concentrations of pyruvate (Yancey and Somero 1978) to ensure that the rate of LDH function can respond to changes in substrate concentration.

The conservation of K_m has previously been demonstrated in vertebrates having widely different body temperatures, as shown by the data in the top frame of Fig. 1. The K_m of pyruvate values for the LDHs of the five sciaenid species are similar to those of other vertebrates, when comparisons are made at all species' physiological temperatures. In addition, there is variation among the LDHs of the sciaenids in the steepness of the K_m versus temperature plots. The red drum, *S. ocellatus*, which appears to encounter the widest range of habitat (= body) temperatures, has the LDH with the lowest sensitivity to temperature. This insensitivity may be an adaptation for conserving appropriate LDH function, *i.e.*, pyruvate binding ability, in a highly eurythermal species like the red drum. The V_{max} of LDH reaction of this species also has the lowest temperature sensitivity, as shown by the comparisons of Arrhenius activation energy values in Table 1. In addition, LDH of the red drum was found to be highly thermostable (Coppes, unpublished obser-

vations), unlike the A_4-LDHs of other teleost fishes (Coppes, Schwantes and Schwantes 1987).

In summary, the A_4-LDHs of the five sciaenid fishes resemble other vertebrate A_4-LDHs in having K_m of pyruvate values conserved at physiological temperatures. The LDHs of the sciaenid species differ, however, in their temperature sensitivities of K_m and V_{max}, with the LDH of the red drum, an extremely eurythermal fish (Holt and Holt 1982), showing the least sensitivity to variations in temperature.

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ASSESSING IMMUNOCOMPETENCE OF RED DRUM (*SCIAENOPS OCELLATUS*)

D. H. Lewis and Peter Thomas[1]

Department of Veterinary Microbiology and Parasitology
College of Veterinary Medicine, Texas A&M University
College Station, Texas 77843

ABSTRACT

Immune responses were manifest in red drum (*Sciaenops ocellatus*) following exposure of the fish to three immunogens, bovine erythrocytes, bovine serum albumin and *Escherichia coli* bacteriophage. These immunogens are frequently used to assess immunocompetence of man and other vertebrates following immunosuppressive treatment. Serologic responses to these materials are proposed as a method for assessing the potential effects of stressors and various other treatments upon host immunity in cultured fish.

INTRODUCTION

Immunity plays a key role in the health and survival of teleosts. Health or disease is the culmination of a sequence of delicately balanced processes involving host, infectious agent and the surrounding environment. Various stressors, including physical environmental factors, nutritional abnormalities and chemicals can profoundly influence the fish's response, not only to other stressors but to infectious agents as well (Anderson, van Muiswinkel and Roberson 1984).

Efforts directed toward assessing the fish's response to various stressors have most often focused upon hormonal or other biochemical alterations in host physiology (Wedemeyer and McLeay 1981). Robertson, Thomas, Arnold and Trant (1987) measured plasma cortisol as a primary endocrine stress response, plasma glucose as an index of metabolic disturbance and plasma osmolality as a measure of osmoregulatory dysfunction in red drum subjected to various stressors. These workers observed increased plasma cortisol and glucose titers during transport of red drum, but recovery to original levels was observed a few hours later. Increasing the rearing density of red drum also induced temporary elevation of plasma cortisol, but had no effect upon plasma glucose or plasma osmolality. They concluded that these parameters could not be used as reliable indices of chronic stress, but a profound and persistent elevation of plasma cortisol in these fish was indicative of an acute stressor such as bacterial infection.

Although it is generally recognized that vertebrate immune responses are compromised as a result of exposure to chronic or acute stressors, investiga-

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[1] The University of Texas at Austin, Marine Science Institute, Port Aransas, Texas 78373-1267

tions on the dynamics of fish immunity following exposure of fish to various stressors have suffered from a lack of standardized assays for immunocompetence. The present report concerns one approach for assessing immunity in red drum. Antigenic stimuli which the fish were not likely to have been previously exposed to were selected to represent:

a) a soluble component to measure precipitins,
b) a component to assess virus neutralizing capabilities and
c) a particulate component to assess agglutinin development.

MATERIALS AND METHODS

Fish

Juvenile red drum (*Sciaenops ocellatus*) were captured in the shipping channel near the University of Texas Marine Science Institute, Port Aransas, Texas. Fish were maintained in a closed recirculating seawater system 25 - 33‰ S at ambient laboratory temperature (20°C) for approximately one month prior to use. Fish were divided into groups of seven fish per treatment. One group of seven fish received 0.9% saline intramuscularly and served as a control. Antigenic materials were administered to fish as described below.

Duplicate studies were conducted in Fall 1985 and Spring 1986.

Antigens

Bovine serum albumin (BSA, Armour Pharmaceuticals Ltd., Chicago) was dissolved in 0.9% saline; bovine blood was collected in 0.01% heparin from animals maintained at the Veterinary Research Park, Texas A&M University and washed five times in 0.9% saline and resuspended to a concentration of 1% w v; bacteriophage MS-2 propagated in *Escheria coli* strain C (ATCC 122) as described by Stashak, Baker and Roberson (1970) were used as antigens in this study. Fish received a single intramuscular injection (0.5 ml) in the dorsal epaxial muscle and serum samples were taken after 5 weeks from the dorsal aorta near the last branchial arch.

Assays

Precipitating antibody was assayed by the agar gel immunodiffusion technique on 10 cm × 10 cm glass plates coated with 1% agarose containing 10 mg bovine serum albumin per ml and 0.05% sodium azide. Two hundred fifty microliters fish plasma was placed in 75 mm wells and after 72 h incubation, the plates were soaked for 10 min in physiologic saline, 10 min in distilled water, and the plates were pressed under a stack of weighted paper towels. The agarose film was dried and stained with Coomassie Brilliant Blue and then destained with acetic acid: methanol: H_2O (5:100:100). Reactions were interpreted as positive when precipitin rings were observed at least 2 mm beyond the edges of the wells.

Hemagglutinins were detected in 96-well microtiter plates (Alexander Corp.) by making doubling dilutions of fish serum in physiologic saline, adding 1% washed bovine blood cells and observing for agglutination reactions after 8 h and 24 h incubation at 4°C.

Phage neutralizing activity was assessed using the agar layer assay method by determining the number of plaque-forming units (PFU) after mixing equal volumes of fish serum and approximately 200 PFU phage and incubating overnight 4°C. A dense suspension of an overnight culture of *E. coli* in tryptone broth was used as the bacterial inoculum in the assay. Generally, incubation of ten assay plates for 5 - 6 h at 37°C was sufficient to produce clearly visible plaques of maximum size for counting. Counting was conducted electronically (BioTran III, New Brunswick, NJ).

RESULTS

Control fish possessed neither BSA precipitins, hemagglutinins nor phage neutralizing antibodies. Other immune responses were specific to the administered antigens. Fish receiving washed erythrocytes developed hemagglutinin titers to a 1:64 serum dilution (1:32 ± 1 unit) and no phage neutralization titers; and fish receiving phage developed plaque reduction titers of approximately 1:128 (± 1 doubling dilution unit) (Table 1).

TABLE 1

Assessment of Immunocompetence of Red Drum

Immunogen	Reaction (14 individuals/group)*
Bovine Serum Albumen (Precipitin reaction)	+*
Bovine Erythrocytes (reciprocal geometric mean hemagglutination titer)	64
Phage Neutralization (reciprocal geometric plague reduction titer)	128

* Serologic reactions were negative in the non-immunized group and cross reactions between the various test groups were not observed.

DISCUSSION

Investigations of the immunocompetence of commercially important fish species have provided the basis for developing an array of vaccines useful in the protection of fish against various disease agents. While it is generally believed that compromised immunity is a component of the generalized stress phenomenon of most vertebrates, most studies of this phenomenon have focused upon assays directed toward hematologic or blood chemistry parameters.

Phage neutralization has been used by various investigators to assess immunocompetence of human beings receiving immunosuppressants (Stashak *et al.* 1970). The present study extends the technique to red drum.

In contrast to results of the present study, BSA does not appear to produce precipitins in some species of fish (Harris 1973). However, in these studies adequate precipitin titers were observed. The red drum does not appear to

produce agglutinins to bovine erythrocytes unless immunized with those erythrocytes.

These studies suggest that serologic assays based upon precipitin and hemagglutinin development and phage neutralization would be adequate for assessing the immunocompetence of red drum in the culture system. Such an assessment would seem useful for ascertaining the effects of various stressors inherent in the aquaculture setting.

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CHANGES IN PROLACTIN CELL SIZE AND CHLORIDE CELL NUMBER IN YOUNG RED DRUM (*SCIAENOPS OCELLATUS*) DURING SALINITY ADAPTATION

Hong Y. Yan[1] and Peter Thomas

The University of Texas at Austin
Marine Science Institute
Port Aransas, Texas 78373

ABSTRACT

Changes in the number of chloride cells in the gill epithelium and the size of prolactin cells in the adenohypophysis of young red drum (*Sciaenops ocellatus*) reared at 28‰ salinity were studied by light microscopy during the course of adaptation to various salinities. After 7 and 21 days exposure, the size of the prolactin cells increased in fish held at 5‰ salinity, whereas a reduction of the cell size was observed at 45‰ salinity. No significant change in the number of chloride cells was observed 7 days after exposure to the different salinities. After 21 days exposure, the number of chloride cells had increased in fish held at 45‰ salinity, whereas a reduction in cell number was observed at 5‰ salinity. The significance of these changes in red drum during adaptation to different salinities was discussed.

INTRODUCTION

The eta cells of the pituitary gland secrete prolactin, which is involved in adaptation of euryhaline teleosts to low salinity (Loretz and Bern 1982). When euryhaline fish move into low salinity seawater there is an increase in the secretion and synthesis of prolactin which subsequently causes an increase in prolactin cell size (Sage and Bern 1971). Chloride cells are the sites of branchial NaCl secretion in euryhaline fish (Karanaky 1986). Exposure to high salinity seawater causes an increase in the number or size of chloride cells in many euryhaline fish (Langdon and Thorpe 1984). Red drum (*Sciaenops ocellatus*) is a euryhaline teleost which has been successfully acclimated to freshwater (Lasswell, Garza and Bailey 1977) and has been collected from salinities as high as 50‰ (Simmons and Breuer 1962). Osmotic adaptation of juvenile red drum (body weight: 1.1-3.8 g; standard length: 32-43 mm) to various salinities has been investigated (Wakeman and Wohlschlag 1983; Crocker, Arnold, DeBoer and Holt 1983); however, morphological changes in the prolactin cells and alterations of the number of chloride cells during adaptation to salinity changes have not been investigated in this species. The purpose of the present study was to monitor changes in the size of prolactin cells and chloride cell number in immature red drum during 3 week exposure to high and low salinity seawater.

[1] Present address: The University of Maryland, Baltimore County Campus, Department of Biological Sciences, Catonsville, Maryland 21228.

MATERIALS AND METHODS

Eighteen immature red drum (body weight: 109-350 g; standard length 9-31 cm), originally reared in captivity in 28‰ seawater, were transferred to 6 recirculating experimental tanks (3 fish tank, 1000 L). The salinities of tanks were adjusted to the test salinities (5, 20, and 45‰; 2 replicates for each salinity) over a period of five days. Throughout the experimental period, fish were fed twice daily with Purina 5 trout chow and water temperature was maintained at 23° ± 1°C. Photoperiod regime was set at 12L/12D. Light intensity was maintained at 7.5 $\mu E/M^2/S$. Fish were sampled before the experiment (control) and after 7 and 21 days (from the end of the 5-day pre-acclimation period) exposure to the test salinities (three fish per treatment at each sampling period). Pituitary glands and the left first gill arches were removed and fixed in Bouin's solution. Tissues were embedded in paraffin and sectioned at 8 μm. The pituitary gland sections were stained with Herlant II tetrachrome (Humason 1979). The diameters (d) of the prolactin cells in the rostral pars distalis were measured with an ocular micrometer under a light microscope by randomly selecting 200 prolactin cells from 10 sections. Since the prolactin cell is spherical in shape, the volumes (V) of the cell were calculated by the formula: $V = 4/3 \times \pi \times (d/2)^3$. Sections of gills were stained with hematoxylin and eosin. The number of chloride cells per unit length (300 μm) of gill epithelium were counted under a microscope following the same protocol for prolactin cells. In order to avoid possible bias, measurement of prolactin cell size and counting of chloride cell number were done blindly, *i.e.* sections were first assigned a random code and later checked with the salinity treatment being given.

RESULTS

Prolactin cells form a compact mass of round cells and lie mainly in the rostral region of the adenohypophysis. After one week exposure, the size of the prolactin cells of the fish held at 5‰ salinity increased about 75% (mean volume: 71.76 μm^3, $P < 0.01$, Fig. 1), whereas the prolactin cell volume of fish at 45‰ salinity decreased approximately 39% (mean volume: 24.60 μm^3, $P < 0.01$, Fig. 1). No significant change in the prolactin cell size was observed for fish held at 20‰ (mean volume: 50.06 μm^3, $P > 0.05$). After 21 days of exposure to the test salinities, there was a 3.4 fold increase in the size of the prolactin cells in fish held at 5‰ salinity (mean volume: 170.6 μm^3, $P < 0.01$). Further reduction of the prolactin cell size was observed in fish held at 45‰ salinity (mean volume: 19.61 μm^3, $P < 0.01$). However, the prolactin cell size of fish at 20‰ salinity (mean volume: 47.56 μm^3) was about the same ($t = 1.55$, $P > 0.13$) as that of control fish (mean volume: 40.47 μm^3, Figs. 1, 2).

Chloride cells in red drum are pear-shaped and mainly located in the interlamellar regions where they are irregularly spaced or form clusters of cells in the primary epithelium. Chloride cells are directly exposed to the external medium through the apical crypts. No significant changes in the number of chloride cells in the gill epithelia were observed in fish after 7 days of exposure to the test salinities (5‰ salinity: 16.33 cells/unit length; 20‰ salinity: 16.75 cells/unit length; 45‰ salinity: 16.25 cells/unit length). A significant and positive correlation ($r = 0.6$, $P < 0.0001$) between the number of chloride cells and salinity was observed after 21 days exposure of fish to the test salinities which can be represented by the following equation: Number of chloride cells per 300 μm = 10.60 + (0.18 × Salinity) (Figs. 3, 4). There was a

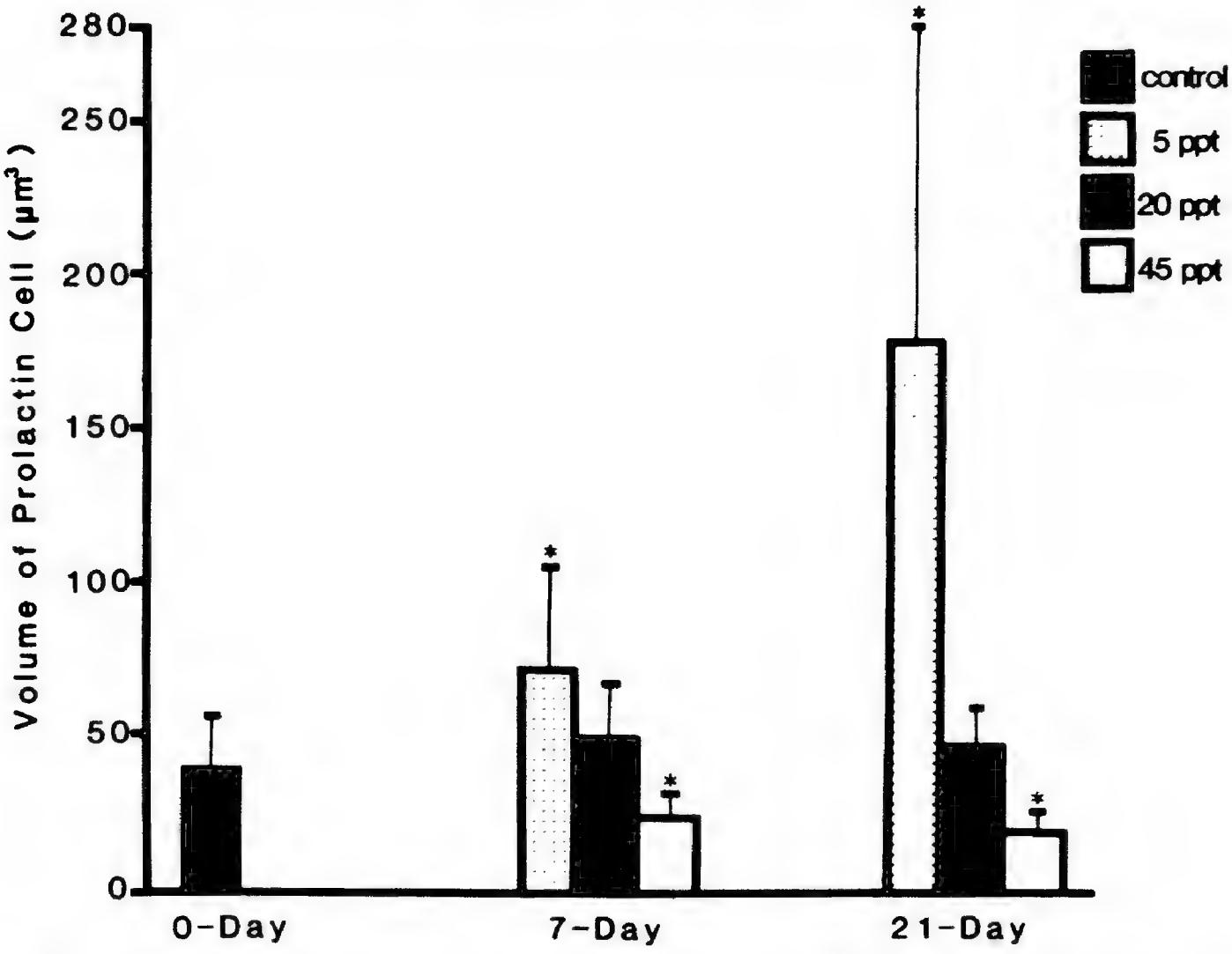

FIG. 1. Effect of salinity change on the size of prolactin cells (mean ± standard deviation) in red drum. Asterisk (*) indicates significantly different from control fish (Day 0, 28‰).

significant decrease in the number of chloride cells in fish exposed to 5‰ seawater (10.80 cells/unit length, $P < 0.01$) and an increase in fish exposed to 45‰ (18.25 cells/unit length, $P < 0.01$). After 21 days exposure of fish to the test salinities, a significant and negative correlation ($r = -0.55$, $P < 0.0006$) between the number of chloride cells and volume of prolactin cell was observed. The relationship can be represented by the following equation: number of chloride cells per 300 μm = 16.84 − (0.02 × prolactin cell volume).

DISCUSSION

The results of the present study with red drum confirm earlier findings with other euryhaline species, *e.g. Poecilia latipinna, Fundulus heteroclitus* and *Mugil cephalus* (Ball and Pickford 1964; Ball, Olivereau, Slicher and Kallman 1965; Abraham 1971) that chloride cell number increases in high salinities and prolactin cell size increases in low salinities. No changes were observed in the number of chloride cells during the first week of exposure to the different salinities, although blood serum osmolalities of juvenile red drum

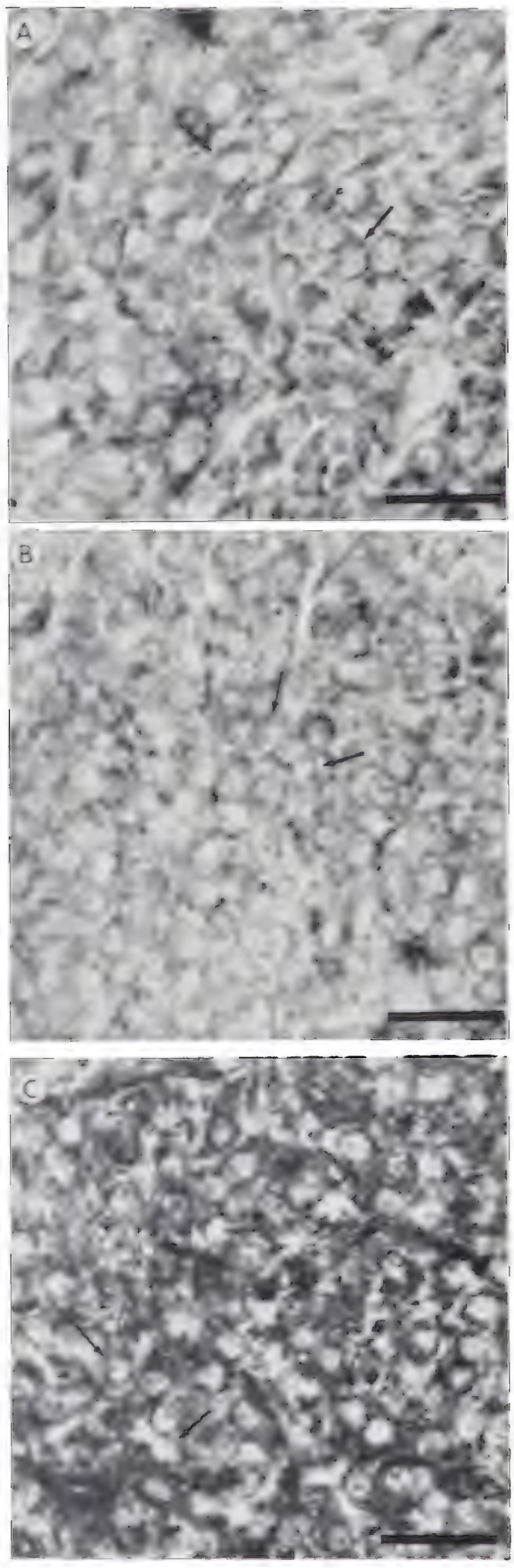

FIG. 2. Size of prolactin cells in red drum after 21 days of exposure to different salinities: (A) 5‰; (B) 20‰; (C) 45‰. Arrows indicate the prolactin cells. Bar equals 20 μm.

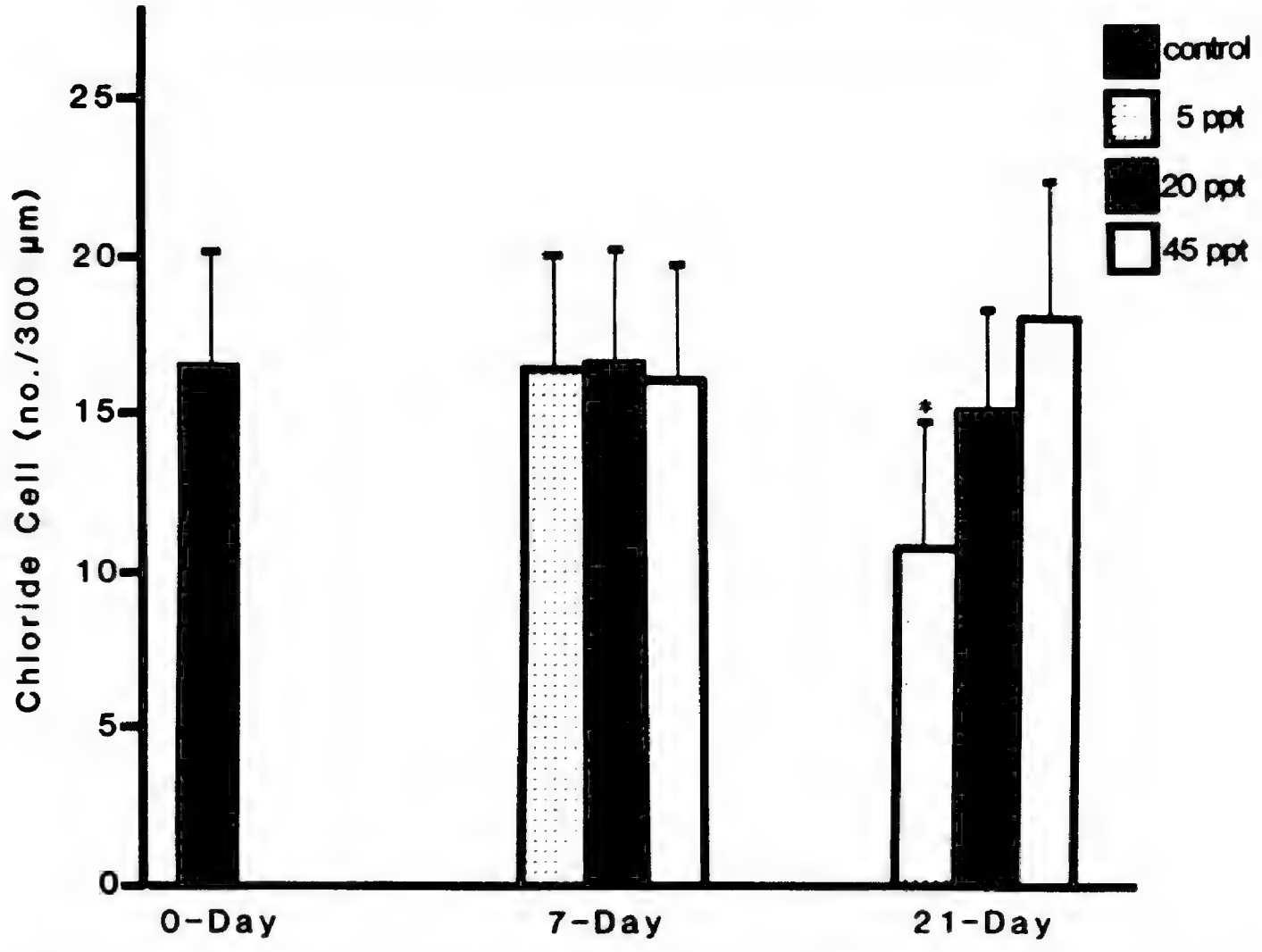

FIG. 3. Effect of salinity change on number of chloride cells (Mean ± standard deviation) in gill epithelia of red drum. Asterisk (*) indicates significantly different from control fish (Day 0, 28‰).

were reported to stabilize at new levels within 24-48 hours after abrupt transfer to new salinities (Crocker *et al.* 1983; Wakeman and Wohlschlag 1983). Apparently chloride cells in red drum respond more slowly to salinity fluctuations than in some other species (Foskett, Bern, Machen and Conner 1983). It has been suggested that morphological changes of chloride cells following salinity transfer are under hormonal or neural control or indirectly induced by increased body fluid osmolality (Foskett *et al.*, *op. cit.*). In contrast, prolactin cell volume in red drum changed rapidly in response to salinity changes. The same phenomenon was reported in seawater adapted medaka (*Oryzias latipes*) five days after transfer to freshwater (Nagahama 1973). In medaka, the prolactin cells showed obvious ultrastructural changes only 1 hr after transfer from seawater to freshwater, although significant changes were not apparent by light microscopy until 5 days after transfer (Nagahama 1973). Previous studies with hypophysectomized fish have demonstrated that prolactin is essential for survival of many euryhaline fish in low salinity seawater (Ball 1965). Prolactin appears to exert an osmoregulatory role in freshwater largely through sodium retention and prolactin secretion shows a marked decline in teleosts following seawater transfer (Loretz and Bern 1982). Although serum prolactin concentration was not measured in the present

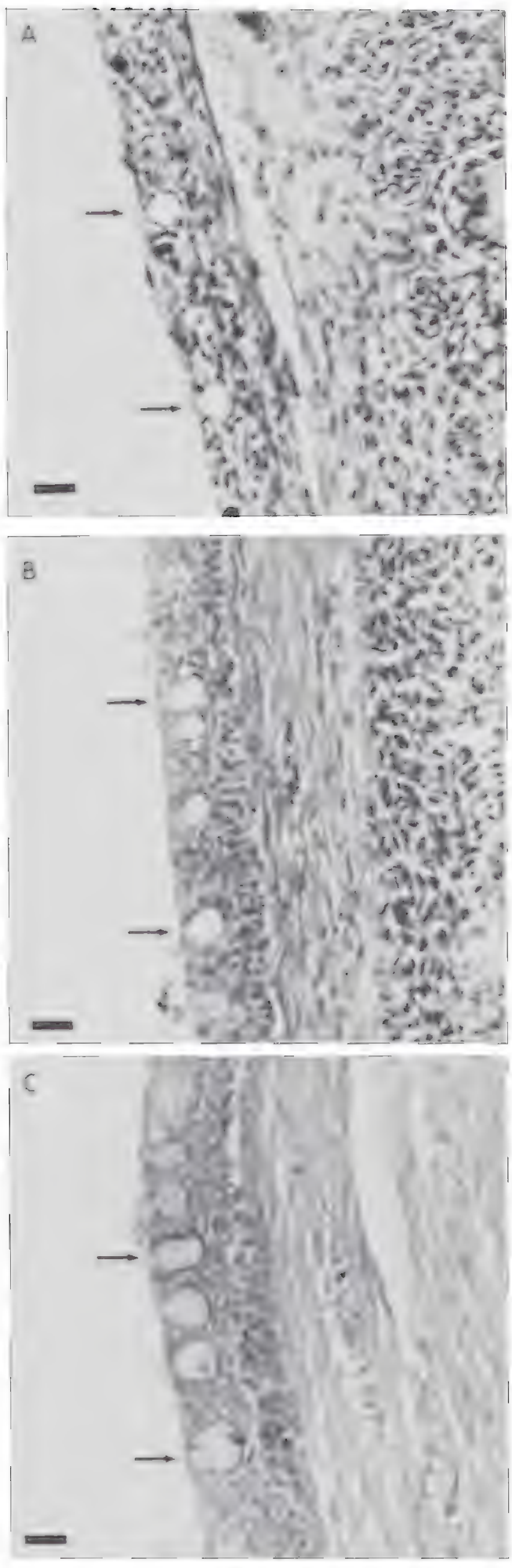

FIG. 4. Number of chloride cells in the gill epithelia of red drum after 21 days of exposure to different salinities: (A) 5‰; (B) 20‰; (C) 45‰. Arrows indicate the chloride cells. Bar equals 20 μm.

study, the substantial reduction in prolactin cell volume of red drum kept at the higher salinity may indicate a reduction of prolactin secretion. Repeated prolactin injections into seawater-adapted *Tilapia* caused substantial inhibition of chloride secretion, and induced a dedifferentiation of chloride cell (Foskett, Machen and Bern 1982), which might explain the significant and negative correlation between prolactin cell volume and the number of chloride cell observed in the present study.

The results of the present study may be of practical significance in mariculture and fisheries management. For example, the long time required for chloride cell number in red drum to adjust to salinity changes is of importance to fish culturists wishing to transfer red drum from high to low salinities. Further, measurement of chloride cell number and prolactin cell size in wild-caught fish could, under certain circumstances, provide useful information on fish migration from one salinity to another and residency time of fish at a particular salinity.

ACKNOWLEDGMENTS

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A DIET INDUCED DISEASE IN COMMON SNOOK, *CENTROPOMUS UNDECIMALIS*

M.E. Clarke, W. Sheldon, K. Dowd and M. Schmale

Rosenstiel School of Marine and Atmospheric Science
University of Miami
4600 Rickenbacker Causeway
Miami, Florida 33149

ABSTRACT

High mortalities occurred in juvenile common snook, *Centropomus undecimalis*, fed a diet of chopped shrimp at the University of Miami Experimental Fish Hatchery. Histological examination of these fish identified no pathogen. In order to determine the role of diet in determining the growth rate and survival of juvenile snook, juveniles were fed three different diets for 39 days and their growth and survival was assessed. The diets were a prepared gelatin food mix containing no shrimp, chopped shrimp and chopped shrimp with a vitamin mixture added. The average mortality in replicate groups fed only chopped shrimp was 30%, while the average mortality of juveniles fed chopped shrimp and vitamins and a gelatin food mix were 5 and 6% respectively. Fish fed chopped shrimp with vitamins showed the highest growth rate.

INTRODUCTION

Most reviews of the general nutritional requirements of fish have concentrated on fishes with a long history of aquaculture such as salmonids, catfish, carp and tilapias (NRC 1973, NRC 1977, NRC 1981 and Jauncey and Ross 1982). There is now an interest in the aquaculture of other species such as snook, sea trout and redfish, but the nutritional requirements of these fish are not well known. Nutritional inadequacies can lead to poor growth and high mortalities of fish in culture.

The importance of determining nutritional requirements of fish during pilot aquaculture was demonstrated by high mortalities of juvenile common snook, *Centropomus undecimalis*, probably resulting from vitamin inadequacy in the diets. Recently metamorphosed snook reared at the Experimental Fish Hatchery at the University of Miami during the 1985 spawning season were fed a diet composed exclusively of chopped penaeid shrimp. Groups of snook raised on this diet grew rapidly, but experienced extremely high mortalities between 50 and 90 days after hatching. Total mortality during this time period was as high as 100% in grow-out tanks. Mortalities were consistently preceded by the onset of several changes in the behavior and appearance of affected fish. The two most frequently manifested symptoms were lateral rolling, usually exhibited when the animal was at rest,and sporadic muscle twitches or in some cases an "s" posture when at rest. These changes suggested a progressive loss of equilibrium and muscle control in

these fish. Histological examination of a small number of snook sampled from the tanks indicated that consistent pathological changes were not present in any tissues.

MATERIALS AND METHODS

During the 1986 spawning season the effect of a variety of diets on growth and survival of juvenile snook was tested. Nine tanks were stocked with 48 juvenile snook at 54 days after hatching (mean size 22.2 mm). Fish were fed live adult artemia until day 60 when groups were weaned onto their respective experimental diets. Fish were held in 380 liter tanks with daily 25% water exchanges until day 75 after which a constant flow of one liter per minute was established. Tank temperatures were maintained between 25°C and 27°C throughout the experiment. Three diets were tested (Table 1). The diets tested were gelatin bound diet mix, chopped penaeid shrimp, and chopped penaeid shrimp supplemented with a vitamin mixture (Table 2). Fish were fed the appropriate diet at approximately 15% of body weight per day. Symptoms of disease and mortalities were noted daily. Fish were sampled at 80 days and at the end of the experiment when the fish were 93 days old.

RESULTS AND DISCUSSION

The average mortality in replicates fed only chopped shrimp was 30% while the average mortality of juveniles fed chopped shrimp and vitamins and the prepared gelatin mix were 5 and 6% respectively. The most common symptoms observed prior to mortality were identical to those observed in previous culture experiments. In some cases subcutaneous hemorrhaging and dermal lesions were observed. Fish fed chopped shrimp supplemented with vitamins showed the highest growth rate of 6.4 mm per day. The fish fed chopped shrimp and gelatin bound diet exhibited growth rates of 5.7 mm per day and 4.8 mm per day respectively. The lower growth of gelatin bound diet may have been due to a reduced palatability of this diet.

The importance of specific vitamins has been demonstrated in many fish and a number of deficiency diseases have been described (Millikin 1982, NRC 1981) and some symptoms resembled those described for fish identified as having pyroxidine deficiency. The symptoms of juvenile snook fed chopped shrimp resented in this study closely resembled the symptoms reported for thiamine deficiency (Woodbury 1942, Halver 1953). The symptoms of thiamine deficiency are described as a melanotic appearance, subcutaneous hemorrhaging, poor appetite and growth, muscle atrophy, loss of equilibrium, and convulsions. The presence of thiaminase in anchovy has been postulated as the cause of thiamine deficiency symptoms in yellowtail, *Seriola guingueradiata*, (Ishihara, Hara, Yagi and Yasuda 1978). Addition of 1 mg per kilogram of body weight per day prevented signs of thiamine deficiency. Injection of 2 mg thiamine yielded a 90% recovery by trout within 2 days (Woodbury 1942). The vitamin supplemented shrimp diet and gel bound diet contained 6.9 mg thiamine per kilogram of feed. Therefore, snook fed these two diets obtained approximately 1 mg thiamine per kilogram body weight at the ration of 15% of body weight and this may have been the cause of the

TABLE 1

Composition of diets tested

Diet	Component	Weight g/l
Gelatin bound mix	Flounder	316.8
	Squid	158.4
	Rangen soft moist salmon pellets	158.4
	Gelatin powder	190.3
	Filtered seawater	174.3
	Vitamin mix	0.6
	Choline chloride	0.6
	Paprika (for additional carotenoids)	0.6
Penaeid shrimp	Penaeid shrimp with no additives	1000.0
Penaeid shrimp w/vitamins	Penaeid shrimp	998.8
	Vitamin mix	0.6
	Choline chloride	0.6

TABLE 2

Composition of vitamin supplement mixture

Vitamin	Weight mg/kg of diets
A	12.4
D	0.9
E	151.4
C	309.7
Myo-Inosito	34.4
K (Menadione)	15.5
α – Aminobenzoic acid	34.4
Niacin	29.3
Riboflavin	6.9
Pyridoxine HCl	6.9
Thiamine HCl	6.9
Ca-Pantothenate	20.6
Biotin	0.1
Folic Acid	0.6
B12	0.01

lower mortality of fish on these diets. Shrimp are generally lacking in a variety of vitamins. Supplementation with a vitamin mix may have eliminated other vitamin deficiencies as well. Further studies on the thiaminase activity of shrimp and treatment of fish with specific vitamins rather than mixed vitamin supplements may identify specific deficiency syndromes.

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BIOPHYSICAL MODEL OF OSMOREGULATION AND ITS METABOLIC COST IN RED DRUM

J. D. Bryan, K. D. Ham and W. H. Neill

Department of Wildlife and Fisheries Sciences
Texas Agricultural Experiment Station
and
Texas A&M University
College Station, Texas 77843

ABSTRACT

Published information on osmoregulatory physiology in euryhaline teleosts was integrated with biophysical principles to construct a mechanistic model of osmoregulation and its metabolic cost in red drum (*Sciaenops ocellatus*). Osmoregulatory processes represented in the model are active and passive branchial ion flux, branchial osmotic water flux, active ion uptake in the gut, osmotic water uptake in the gut, urinary water and ion loss, and ion reabsorption from the urine. Osmoregulatory cost is calculated on the basis of electro-osmotic work required for active ion transport in the gill, gut, and kidney. An appropriately refined variant of the model produced water and ion fluxes consistent with those reported for euryhaline teleosts; moreover, this model accurately represented the published effects of salinity on standard metabolic rate of red drum.

INTRODUCTION

Red drum survive in coastal and inland waters that have greatly divergent concentrations of total dissolved solids ("salinity") and specific ions. Evaluation of such waters' potential for red drum (*Sciaenops ocellatus*) aquaculture would be facilitated by ability to predict the fish's energy cost for osmoregulation. To this end, we have developed a model of osmoregulation and its bioenergetic cost in red drum. The model is based on thermodynamic relationships and on published observations of osmoregulatory physiology in various teleosts.

MODEL STRUCTURE

The osmoregulation model conformed generally with textbook descriptions (*e.g.* Smith 1982) of osmoregulatory physiology in euryhaline teleosts. Osmoregulatory processes represented in the model are active and passive branchial ion flux, branchial osmotic water flow, active ion uptake in the gut, osmotic water uptake in the gut, urinary water and ion loss, and ion reabsorption from the urine. Ion fluxes are modeled only for Na^+, K^+, and Cl^-, which are relatively abundant and/or have relatively high membrane permeability; osmotic water flow is modeled on the basis of Na^+, K^+, Cl^-, Ca^{++}, Mg^{++}, and $SO4^{--}$ concentrations. The model calculates osmoregulat-

ory cost as the rate of oxygen consumption required for maintenance of ion and water balance.

Balance among the water fluxes in the model is established by requiring that osmotic influx across the gut and loss in the urine combine with branchial osmotic flux to produce no net water flux between the fish and the surrounding medium. Water influx in the gut involves active absorption of ions from ingested water in the anterior section of the gut; this reduces osmolarity of the fluid passing to the rear section of the gut so that water can move osmotically down the chemical potential gradient into the blood. Glomerular filtration in the kidney generates a loss of water and ions from the blood; subsequent reabsorption of ions and water from the urine reduces renal loss.

Water balance requires that water influx equal water efflux but does not dictate the magnitude of offsetting fluxes. In the absence of empirical data, we began with the simple assumption that in media hyperosmotic to the blood, red drum establish water balance by drinking to offset the branchial osmotic efflux rate and some minimum urine flow rate. In hypoosmotic environments the drinking rate was assumed to be zero and the urine flow rate equal, but opposite in sign, to the branchial osmotic influx rate.

After establishing water balance, the model determines gut and kidney ion flux rates and active ion flux rates at the gills such that balances are established for Na^+, K^+, and Cl^-. The rate and direction of active branchial transport for each ion is that value producing a mass balance among active and passive branchial ion fluxes and ion fluxes across the gut and kidney. Passive branchial ion fluxes are computed by the Nernst-Planck equation (Kirschner 1982), using the solution given by the Goldman current equation (Koch 1970):

$$Jp_i = \frac{-p_i A_g \frac{zF}{RT} Vg \left[Cw_i - Cb_i e^{\frac{zF}{RT} Vg} \right]}{\left[1 - e^{\frac{zF}{RT} Vg} \right]} \qquad (1)$$

where:

Jp_i = passive flux for ith ion
p_i = membrane permeability for the ith ion
A_g = branchial exchange area
z = valence
F = Faraday constant
R = gas constant
T = absolute temperature
Cw_i = concentration of the ith ion in the water
Cb_i = concentration of the ith ion in the blood

and Vg, the transepithelial potential, is approximated by the Goldman voltage equation (Goldman 1943):

$$Vg = \frac{RT}{F}\log_e \left[\frac{\Sigma \left[u_i^+ Cw_i^+ + u_i Cb_i\right]}{\Sigma \left[u_i^+ Cb_i^+ + u_i Cw_i\right]}\right] \quad (2)$$

where:

u_i^+ = permeability relative to Na^+ of the ith cation
u_i = permeability relative to Na^+ of the ith anion
Cw_i^+ = concentration in the water of the ith cation
Cb_i^+ = concentration in the blood of the ith cation
Cw_i^- = concentration in the water of the ith anion
Cb_i^- = concentration in the blood of the ith anion.

Computation of branchial osmotic water flux is based on the definition of osmotic pressure (Cooney 1976):

$$Qw_g = K_g A_g RT \left[\Sigma \left[Cb_i - Cw_i\right]\right] + K_g A_g dP \quad (3)$$

where:

Qw_g = branchial osmotic water flux
K_g = permeability of gills to water
dP = hydrostatic pressure difference between blood and water.

Membrane permeabilities to water are represented as a negative exponential function of salinity, reflecting the change in permeability with respect to Ca^{++} found by Wendelaar Bonga, Lowik and van der Meij (1983). To determine ion and water flux rates across the gut, the model calculates the total ion concentration of ingested water in the rear section of the gut which would generate the necessary osmotic influx from the gut fluid into the blood. The rate of ion absorption from the anterior gut is that necessary to produce this concentration. Osmotic water flux from the gut (see equation 3) is given by:

$$Qw_s = K_s A_s RT \left[\Sigma \left[Cb_i - Cs_i\right]\right] \quad (4)$$

where:

Qw_s = osmotic water flux across the gut
K_s = permeability of the gut to water
A_s = exchange area of the gut for water flux
Cs_i = concentration in the gut of the ith ion.

Ion reabsorption is treated as a set of first-order reactions. This assumes negligible absorption rates for all ions except Na^+, K^+, and Cl^-, that the fraction of ingested water absorbed is constant, and that Na^+, Cl^-, and K^+ are absorbed in proportion to their concentrations in ingested water. The rate constant (r) for these reactions is determined as follows. Applying conservation of mass for ingested water in the anterior gut gives:

$$Cs_i = Cw_i - \frac{Ql_i}{Vw} \tag{5}$$

where:

Ql_i = absorption rate for ith ion
Vw = drinking rate.

Rearranging equation (4) yields the total ion concentration in the rear section of the gut (ΣCs_i). Substituting Cs_i from equation (5) into ECs_i and rearranging leads to:

$$r = \frac{Qw_s}{(1 - fs)\left[Cw_{Na} + Cw_{Cl} - Cw_k\right]}\left[\frac{Qw_s}{K_s A_s RT} + \left[\Sigma\left[Cw_i - Cb_i\right]\right]\right] \tag{6}$$

$$Ql_{Na} = r\,Cw_{Na} \tag{7}$$

$$Ql_{Cl} = r\,Cw_{Cl} \tag{8}$$

$$Ql_k = r\,Cw_k \tag{9}$$

$$Vw = \frac{Qw_s}{(1 - fs)} \tag{10}$$

where:

r = absorption rate constant
Ql_{Na} = sodium absorption rate
Ql_{Cl} = chloride absorption rate
Ql_k = potassium absorption rate
Cw_{Na} = concentration of sodium in water
Cw_{Cl} = concentration of chloride in water
Cw_k = concentration of potassium in water
fs = fraction of ingested water that is not absorbed.

Ion reabsorption and loss in the kidney are calculated from the glomerular filtration rate by assuming that a constant fraction of ions (Na^+, K^+, and Cl^-) is reabsorbed from the urine, giving:

$$Ql_{ki} = Qw_k Cb_i(1 - fk) \tag{11}$$

$$Ql_{ui} = -Qw_k Cb_i fk \tag{12}$$

where:

Ql_{ki} = renal reabsorption rate for the ith ion
Ql_{ui} = urinary loss rate for the ith ion
Qw_k = glomerular filtration rate
fk = fraction of ions in the ultrafiltrate not reabsorbed

and Qw_k, the glomerular filtration rate, is given by

$$Qw_k = Qw_u + K_u A_u RT \left[\left[\Sigma Cb_i\right] \left[1 + \frac{1}{fb} - \frac{fk}{fb} - fk\right]\right] \quad (13)$$

where:

Qw_u = urine flow rate
k_u = tubule permeability to water
A_u = water reabsorption area
fb = (renal blood flow rate − Qw_k)/Qw_k.

Finally, osmoregulatory cost is calculated as the sum of active ion transport costs in the gill, kidney, and gut. Costs are determined in each case from the electro-osmotic work required to transport ions up their respective chemical and electrical potential gradients (Williams, Mattice and Williams 1978):

$$QO_2 = QI_A c \left[RT \log_e \frac{Ci}{Co} + zF\,Vc\right] \quad (14)$$

where:

QO_2 = oxygen consumption rate
QI_A = active transport rate
Ci = ion concentration of fluid receiving ions
Co = ion concentration of fluid losing ions
c = caloric equivalent
Vc = voltage between Ci and Co (see equation 2).

Assuming that ions passively diffuse into the blood after they are actively transported from the ingested medium or urine into the interstitial tissue surrounding the capillaries, the tissue ion concentration is given by:

$$Ct_i = Cb_i + \frac{QI_A}{D_i A_i} \quad (15)$$

where:

D_i = diffusion coefficient
A_i = capillary exchange area.

Then, in equation 14,

$$Ci = Ct_i \quad (16)$$

for the gut and kidney.

MODEL EXECUTION

The model was executed for salinities ranging from 1 to 50‰, with ionic ratios like those of Galveston seawater (Howe 1981). Parameter estimates were made for a 100 g red drum with blood concentrations of ions held constant at the values measured by Sulya, Box and Gunter (1960).

MODEL PERFORMANCE AND REFINEMENT

Model performance was evaluated by comparing the relationship between predicted osmoregulatory cost and salinity with that between standard metabolic rate and salinity observed for 73-197 g red drum by Wakeman and Wohlschlag (1983). To facilitate the comparison, we translated predicted osmoregulatory costs to predicted standard metabolic rates by adding to all cost values the constant necessary to equalize predicted and observed salinity minima of standard metabolic rate. The necessary constant was 112 mg O_2 $kg^{-1}hr^{-1}$; this we assume represents non-osmoregulatory standard metabolism of red drum under the experimental conditions of Wakeman and Wohlschlag (1983).

Oxygen consumption rates predicted by the model agreed well with those measured for red drum in freshwater and at 20‰ and above. However, the model considerably underestimated osmoregulatory cost in the zone between the isoosmotic salinity (about 11‰ — Wakeman and Wohlschlag 1983) and freshwater. The model (henceforth, model A) seemed to underestimate drinking rate under isoosmotic conditions, suggesting that red drum in isoosmotic media have a non-zero nominal drinking rate and an associated elevation in urine flow rate.

Model variants incorporating alternative forms of a nominal drinking rate function were developed to determine water flux rates required by the model to generate the metabolic rates reported by Wakeman and Wohlschlag (1983). The first (model B1) established a minimum drinking rate similar to that of eels in fresh water (Maetz and Skadhauge 1968). This model reduced the underestimation of metabolic rate in media between freshwater and 20‰ but overestimated oxygen consumption in freshwater. The second model (model B2) assumed that the nominal drinking rate decays to zero in freshwater (Shehadeh and Gordon 1969) and increases to balance branchial water flux and urinary water loss in hyperosmotic media. Under this model, gut water uptake rate is:

$$Qw_g = Q_{g\backslash} - \beta 1 \left[\exp\left[k_r Qw\right] - 1\right] \tag{17}$$

when $Qw \geq 0$ (*i.e.* during branchial water influx), and gut water uptake rate is:

$$Qw_g = Q_{g\backslash} + \beta 1 \left[\exp\left[-k_r Qw\right] - 1\right] \tag{18}$$

when $Qw < 0$ (*i.e.* during branchial water efflux) where:

$Q_{g\backslash}$ = nominal gut uptake rate
$\beta 1$ = exponential parameter
k_r = exponential rate constant.

Urine flow rate is calculated as:

$$Qw_u = Qw_g - Qw \tag{19}$$

while requiring that if $Qw_u < Qw_u min$ then

$$Qw_u = Qw_u min \tag{20}$$

and

$$Qw_s = Qw - Qw_u \tag{21}$$

Although this model predicted observed oxygen consumption rates better than models A or B1, it still underestimated the rate at 10‰. The use of a higher value of the nominal drinking rate improved the model's fit at 10‰ but caused a shift of the minimum oxygen consumption rate to higher salinities and caused the fit at 20‰ and at salinities less than 10‰ to deteriorate. These problems were eliminated by invoking an abrupt increase in drinking rate at 10‰ which returned rapidly to the value predicted by the exponential drinking rate function on either side of 10‰. However, it is difficult to imagine such an abrupt change in drinking rate except in response to an abrupt change in water balance.

Consequently, we tried a third model variant (model C) in which urine flow is at a minimum in hyperosmotic media and increases rapidly as the medium becomes hypoosmotic, to a maximum in freshwater. The resultant, excessively high urine flow rate in slightly hypoosmotic media causes a compensatory elevation in drinking rate. Drinking rate then declines with decreasing salinity as branchial and urinary water fluxes equalize. This function was implemented by requiring that changes in urine flow rate with respect to changes in branchial water influx rate be proportional to the difference between the maximum urine flow rate and the actual urine flow rate, *i.e.* when $Qw < 0$:

$$Qw_u = Qw_u max - \beta 2 \exp\left[-k_{ru} Qw\right] \tag{22}$$

and

$$Qw_s = Qw_u - Qw \tag{23}$$

where:

$Qw_u max$ = urine flow rate in freshwater
$\beta 2$ = exponential parameter
$-k_{ru}$ = exponential rate constant.

This function provided good agreement between predicted and observed results but shifted the minimum oxygen uptake rate to a salinity near 12‰. While this shift is not refuted by Wakeman and Wohlschlag's (1983) data for red drum, it is not in agreement with the trend exhibited by speckled seatrout (Wohlschlag and Wakeman 1978) and requires a drinking rate much lower than those of other teleosts in isoosmotic media. In order for the model to generate a minimum osmoregulatory cost at other than the isoosmotic concentration, drinking rate must be non-zero at the isoosmotic concentration. Consequently, we combined models B2 and C to form model D, which incorporated both a nominal drinking rate function and a switching function

for renal activity. This model generates metabolic rates very near observed values (Fig. 1); also, it predicts transepithelial potentials (Fig. 2) and rates of water and ion flux (Figs. 3 and 4) similar to those observed in other teleosts. Values of variables and parameters for model D are listed in Table 1.

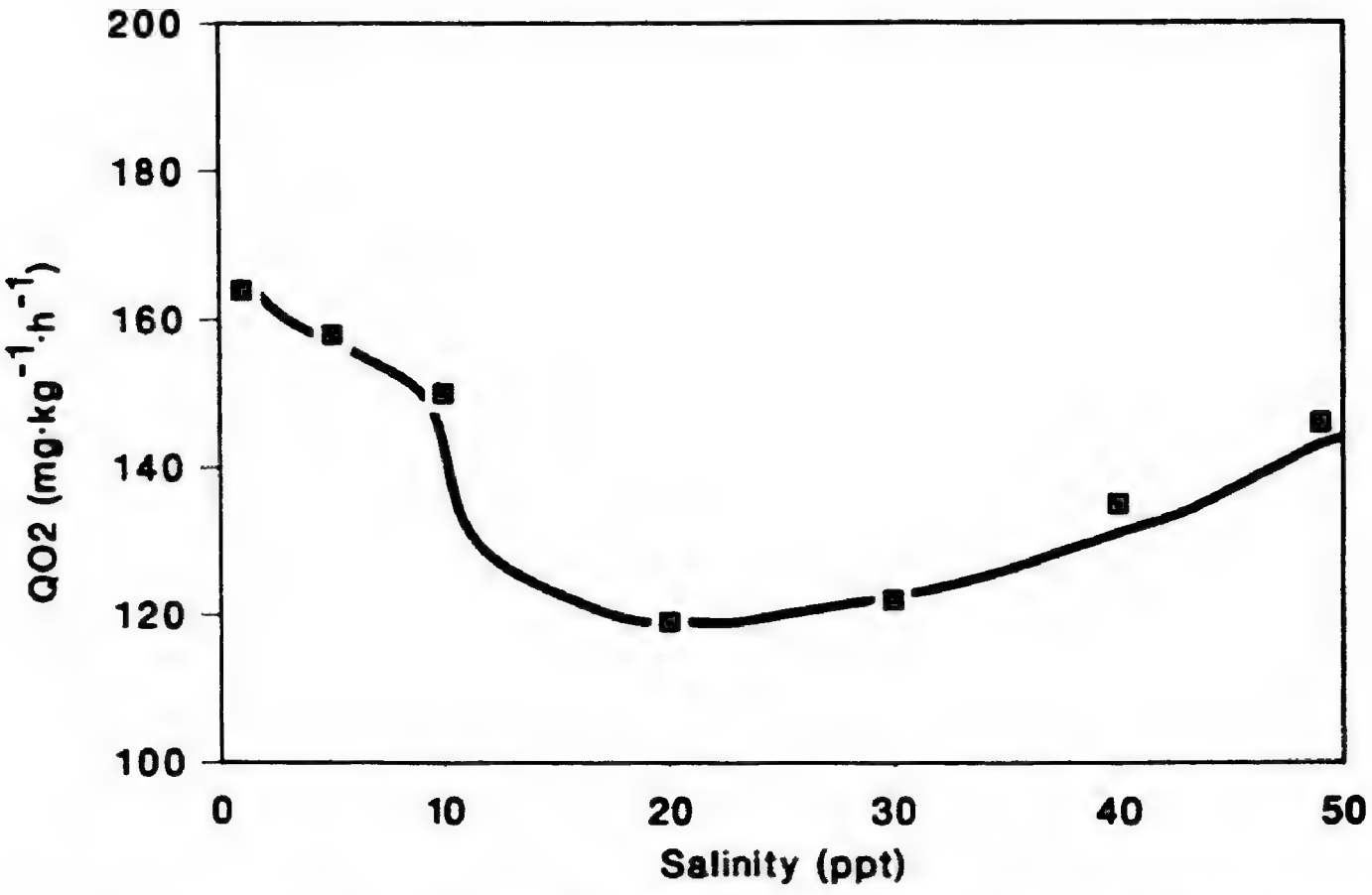

FIG. 1. Standard metabolic rates of red drum as a function of salinity. Closed squares indicate measured values (Wakeman and Wohlschlag 1983); solid line indicates values simulated with the final osmoregulation model (Model D).

CONCLUSION

The last version of the model (model D) we offer as a logically consistent and sufficient set of quantitative hypotheses about osmoregulation and its metabolic cost in red drum. Invoked mechanisms and parameter values are not in violation of those established for other teleosts, and osmoregulatory cost as a function of salinity agrees with that measured experimentally for red drum by Wakeman and Wohlschlag (1983). Validation of the underlying model and associated water flux rates will require both osmoregulatory process and metabolic rate studies in red drum, particularly at the transition from hypo- to hyperosmotic environments and in media with ionic compositions unlike those of seawater.

ACKNOWLEDGMENTS

We thank Dr. Steve Kelsch for comments on the manuscript and help in preparing the figures. This work was supported by a grant from the Texas Advanced Technology Research Program, through Texas Agricultural Experiment Station Project H-6295.

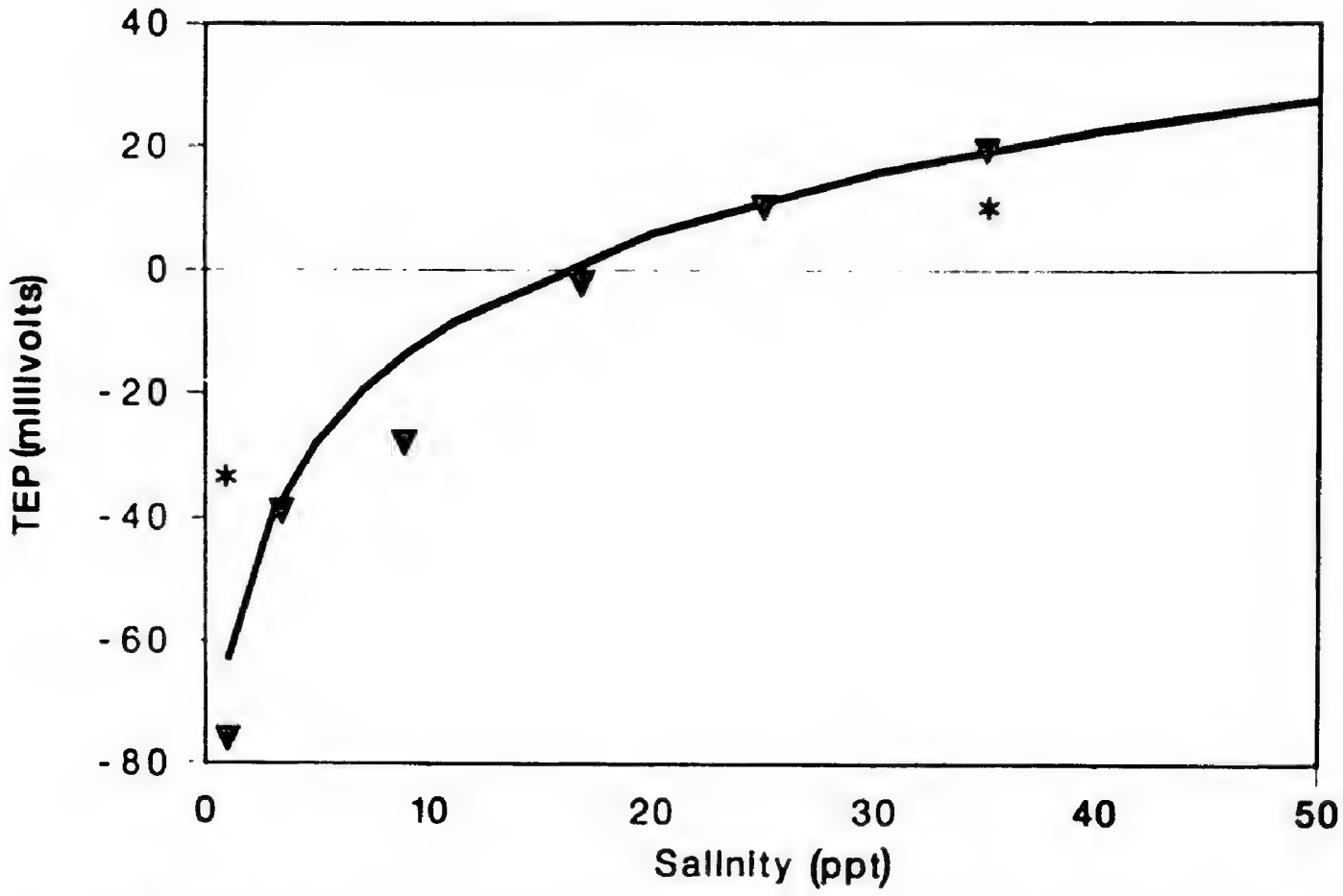

FIG. 2. Branchial transepithelial potential (TEP) as a function of salinity. Solid line indicates values predicted for red drum with equation 2. Measured values are indicated by inverted triangles for flounder (Potts and Eddy 1973) and by asterisks for rainbow trout (Greenwald, Kirschner and Sanders 1974).

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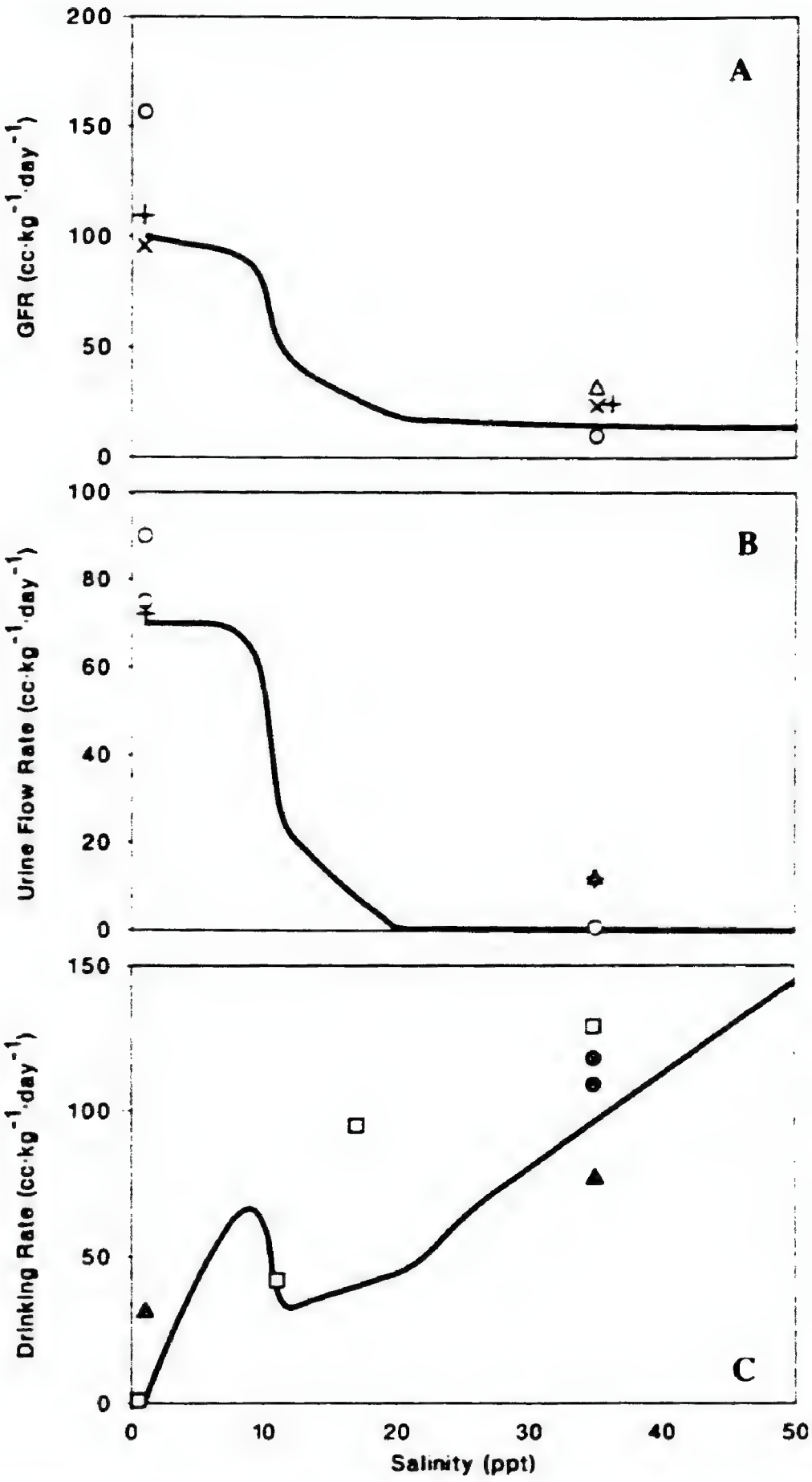

FIG. 3. Glomerular filtration rate (A), urine flow rate (B), and drinking rate (C) as functions of salinity. Solid lines indicate values simulated for red drum with the final osmoregulation model (model D). Measured values are indicated for rainbow trout by open squares (Shehadeh and Gordon 1969) and by open circles (Holmes and McBean 1963), for southern flounder by closed circles (Hickman 1968), for winter founder by open triangles (Renfro 1980), for European eel by closed triangles (Maetz and Skadhauge 1968) and by crosses (Sharratt, Bellamy and Jones 1964), and for representative marine and freshwater teleosts by X's (Smith 1982).

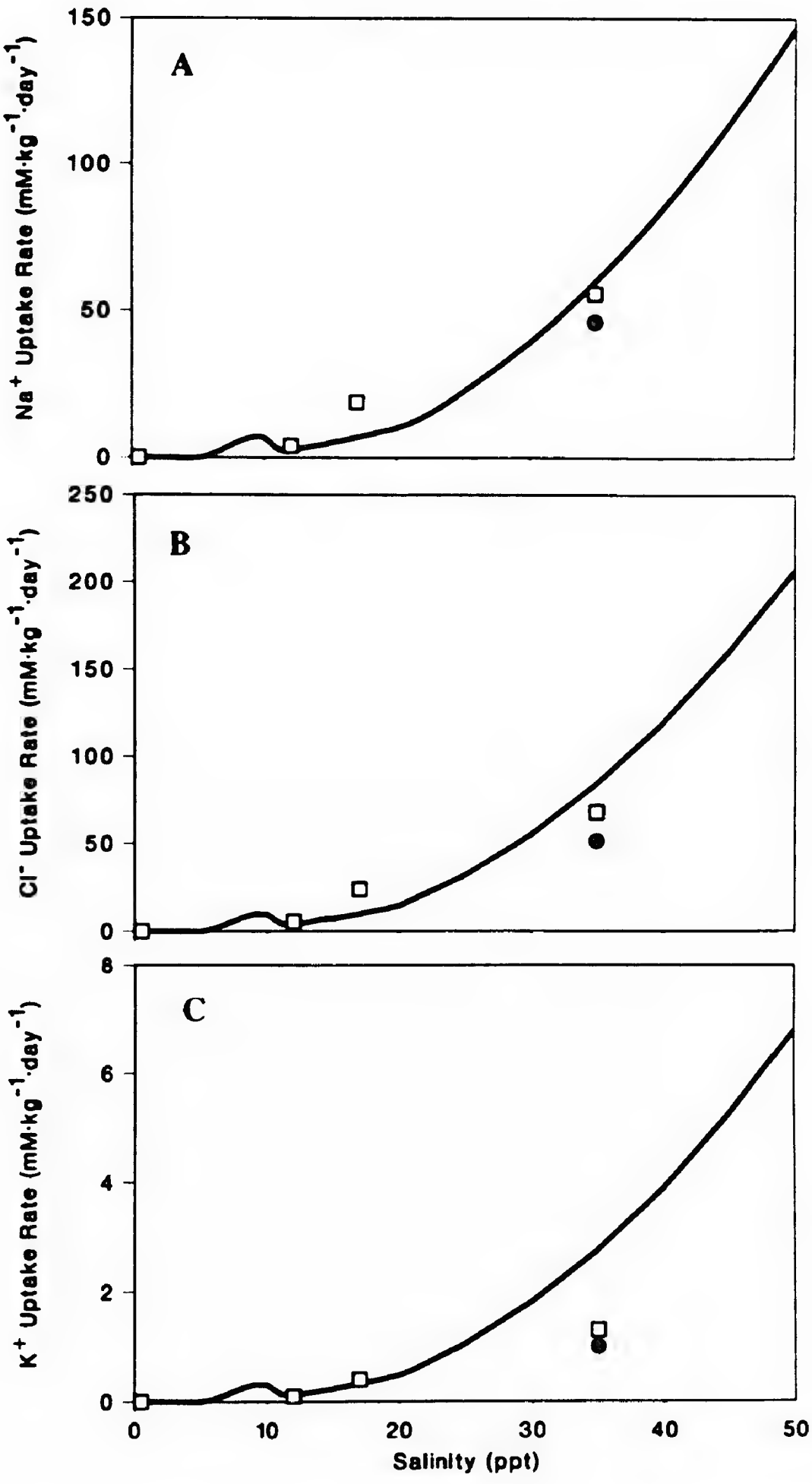

FIG. 4. Gut absorption rates of Na^+ (A), Cl^- (B), and K^+ (C) as functions of salinity. Solid lines indicate values simulated for red drum with the final osmoregulation model (Model D). Measured values are indicated for rainbow trout by open squares (Shehadeh and Gordon 1969) and for southern flounder by closed circles (Hickman 1968).

TABLE 1

Symbolic name and value of variables or parameters used in the final osmoregulation model (Model D)

Variable/Parameter	Value (Units)	[Notes]	Variable/Parameter	Value (Units)	[Notes]
p_i			dP	20,000 (dyne/cm^2)	[8]
Na^+	1.89E-05 (cm/sec)		k_g	2.27155E-14	
Cl^-	$p_{Na} \cdot \pi_{Cl}$ (cm/sec)			(cm^3/(dyne · sec))	[9]
K^+	$p_{Na} \cdot \pi_K$ (cm/sec)	[1]	A_g	300 (cm^2)	[10]
A_g	300 (cm^2)	[2]	f_5	0.2	[11]
T	301 (K)	[3]	f_6	0.01	[12]
Cw_i	Salinity(ppt) · 0.001		k_u	2.27155E-14	
	· (Molecular weight)$^{-1}$			(cm^3/(dyne · sec))	[13]
Na^+	· 0.28 (M/cm^3)		A_u	100 (cm^2)	[14]
Cl^-	· 0.60 (M/cm^3)		f_7	5	[14]
K^+	· 0.022 (M/cm^3)		c	1.54E11 (erg/g O_2)	[15]
Mg^{++}	· 0.029 (M/cm^3)		D_i	p_i	[1]
Ca^{++}	· 0.009 (M/cm^3)		A_j		
SO_4^{--}	· 0.063 (M/cm^3)	[4]	gut	25 (cm^2)	[16]
Cb_i			kidney	100 (cm^2)	[14]
Na^+	1.89E-04 (M/cm^3)		$\dot{Q}_{Ox}$	2.893518E-05	
Cl^-	1.6E-04 (M/cm^3)			(cm^3/sec)	[17]
K^+	8.6E-06 (M/cm^3)	[5]	$\beta 1$	2.893518E-05	
π_i				(cm^3/sec)	[17]
Na^+	1.0		k_r	8679.408 (sec/cm^3)	[17]
Cl^-	0.03		$\dot{Q}_{w_{max}}$	8.101852E-05 (cm^3/sec)	[17]
K^+	2.5	[6]	$\dot{Q}_{w_{min}}$	0.1E-06 (cm^3/sec)	[17]
k	1.362548E-14 + 2.075437E-14		$\beta 2$	5.208334E-05	
	· exp(-6.879839E-02 · Salinity)			(cm^3/sec)	[17]
	(cm^3/(dyne · sec))	[7]	$-kr_s$	-160667 (sec/cm^3)	[17]

[1] Motais and Isia (1972) report a diffusional permeability coefficient for Na^+ at 25°C of 1.4E – 05 cm·sec^{-1}. The value was adjusted by trial and error to 1.89E-05 cm·sec^{-1}. Diffusional permeability coefficients for Cl^- and K^+ were estimated as the ratio of each's permeability to that of Na^+ multiplied by the diffusional permeability coefficient of Na^+.

[2] Gray (1954) and Hughes (1966) estimated that the total lamellar area of typical teleosts averages 5 cm^2·g^{-1} body weight. Hughes (1966) estimated that the exchange area is approximately 60 to 70% of total lamellar area. Therefore, for a 100g red drum 5cm^2·g^{-1}·100g·0.6 = 300cm^2.

[3] Wakeman and Wohlschlag (1983) measured standard metabolic rates at 28°C = 301 K.

[4] Salinities used were obtained from Howe (1981).

[5] Sulya *et al.* (1960).

[6] Potts and Eddy (1973).

[7] Dainty and House (1966) gave pressure-specific osmotic permeabilities of frog skin ranging from 1.97E – 13 to 1.83E – 12 (cm^3·(dyne·sec)$^{-1}$). Permeabilities were adjusted by trial and error to arrive at values around 3.5E – 14 in freshwater and 2.3E – 14 at 12‰. An exponential function describing the change in permeability with salinity was used to reflect the shape of the permeability curve reported by Wendelaar Bonga *et al.* (1983).

[8] Randall (1970) states that normal blood pressure in the ventral aorta of fishes range from 13332.24 to 39996.72 dyne·cm^{-2}. The transmural branchial pressure gradient was approximated at 20000 dyne·cm^{-2}.

[9] Permeability calculated at 12‰. See note 7.

[10] The functional area of the gut for water reabsorption is unknown. Since steady-state water flux across the gill and gut are approximately equal in seawater, the water reabsorptive area in the gut of red drum was approximated by the branchial exchange area.

[11] Shehadeh and Gordon (1969) reported that 80% of ingested water was absorbed from the intestine in rainbow trout.

[12] Hickman and Trump (1969) state that sodium reabsorption in the kidneys of freshwater fishes may be greater than 99.9%. On this basis we assumed 1% urinary loss of all solutes from glomerular filtrate in red drum.

[13] See note 9.

[14] Values of these renal parameters are unknown and were estimated by trial and error.

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[15] Jones (1971) gives the caloric equivalent for oxygen as 1.54E + 11 $erg \cdot g^{-1}$.

[16] Estimated by trial and error.

[17] Fitted parameters for generating desired values in exploratory water-flux functions.

Contributions in Marine Science. (1988) Supp. to Vol. 30:183–189.

PRELIMINARY STUDIES ON THE USE OF CHLOROQUINE AS A SYSTEMIC CHEMOTHERAPEUTIC AGENT FOR AMYLOODINOSIS IN RED DRUM (*SCIAENOPS OCELLATUS*)

D. H. Lewis, Wang Wenxing[1], A. Ayers and C. R. Arnold[2]

Department of Veterinary Microbiology & Parasitology
College of Veterinary Medicine
Texas A & M University
College Station, Texas 77843

ABSTRACT

Since its first description by Brown in 1931, *Amyloodinium ocellatum* has been recognized as an important marine fish pathogen. *Amyloodinium ocellatum* is reported to be primarily a gill parasite which also occurs on the skin (Lawler 1977). Clinical signs associated with infection include petechiae, inflammation and necrosis of gills, secretion of pink-tinted mucous from the opercular cavity, rapid opercular movement, congregation near the water surface, spasmodic gasping, and backflushing of the infected host's gills.

INTRODUCTION

Parasitic portions of the life cycle of *A. ocellatum* begins when the invasive (dinospore) stage infects the fish host. After attaching to gill epithelium, the organism transforms to the parasitic trophont stage, during which time an elaborate attachment apparatus develops. The trophont feeds and remains on the host during maturation, following which the reproductive (tomont) stage detaches, assumes a spherical shape and encysts to form a palmellar stage. A series of divisions begin, the last stage of which results in the production of as many as 256 dinospores (Lom 1981).

In the intensive culture environment, infestations with this parasite are difficult to control because of the parasite's broad tolerance to salinity and temperature (Paperna 1984a). Bath treatments utilizing various copper compounds either alone or in combination with citric acid or formalin have been recommended as control measures for *A. ocellatum* (Dempster 1955, Kingsford 1975, Lawler 1977, Paperna 1984b, Johnson 1984), but none have proven to be entirely satisfactory for controlling *A. ocellatum* infections in cultured red drum (*Sciaenops ocellatus*).

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[1] First Institute of Oceanography, State Oceanic Administration, P. O. Box 98, Quingdao, Peoples Republic of China.

[2] The University of Texas at Austin, Marine Science Institute, Port Aransas, Texas 78373.

Bath exposure as a control measure for amyloodinosis is complicated by the effects of chemotherapeutics upon ecology of the biological filtering system, side effects upon treated fish (Kingsford 1975) and variation in susceptibility of the various strains and parasitic stages of the parasite (Paperna 1984b). Furthermore, the trophont stage of *A. ocellatum* is firmly attached to host gill epithelium and surrounded by a protective mucous coating, which in many instances, renders the parasite refractory to bath treatment.

A study was thus initiated to explore the use of the antimalarial drug, chloroquine as a systemic approach for treating amyloodinosis in red drum.

MATERIALS AND METHODS

Amyloodinium ocellatum used in these studies were axenic cultures originally recovered from red drum, propagated in vitro and maintained in the Department of Veterinary Microbiology and Parasitology, Texas A & M University, College Station, Texas, as described elsewhere (Lewis, Wenxing, Ayers and Arnold 1988).

Experimental fish were juvenile red drum (*S. ocellatus*) weighing approximately 250 g and originated from the Institute of Marine Sciences, University of Texas, Port Aransas, Texas. Fish were maintained in a closed recirculating artificial seawater system (Marine Mix) 25 - 30‰ at ambient temperature (20 C) for approximately one month prior to use.

Approximately 1000 tomonts were harvested from infected cell culture monolayers of dorsal fin cells, washed and resuspended in 10 ml sterile Ott's medium (Ott 1965) and incubated at 25°C for 3 days. Chloroquine susceptibility of the resulting dinospores was assessed by transferring 100 µL dinospore suspension (200- 250 dinospores) into the wells of a 96 well cell culture plate (American Scientific Products, McGaw Park IL 60085-6787). Chloroquine phosphate (Sigma Chemical Company, St. Louis MO 63178) prepared in sterile Ott's medium to yield solutions containing 0.25-, 0.5-, 1.5- and 2.5-µg chloroquine/ml was dispensed in triplicate 100 µl quantities to the dinospore containing wells and incubated at 20°C. The number of motile cells/ IDO cells (expressed as the "percent motility") was determined after 4-, 24-, and 48-hr.

Fish were placed individually into 150 L fiber glass tanks equipped with an external 3.78 L biofilter containing artificial seawater adjusted to 28‰ salinity (Hawaiian Marine Import, Houston Texas 77218) for toxicity-, drug uptake- and tissue-distribution. Chloroquine phosphate was administered orally by placing the drug in gelatin capsules, the capsules surrounded and immobilized in squid tissue and the preparations fed individually to experimental fish.

To assess potential drug toxicity, fish received 5 to 40 mg in 5 mg/kg increments and 100 and 200 mg chloroquine phosphate/kg body weight, 9 fish per treatment. Daily observations were made for 30 days, at which time fish were necropsied for gross signs of toxic effects.

To estimate drug levels in gill mucous 24 hr after administration, fish were divided into groups of 9 fish group and the respective groups each received 10, 40, 60, 100, and 200 mg drug/kg body weights. Twenty four hr after administering drug, gill mucous was harvested with a spatula, transferred to 5 ml preweighed glass beakers and dried to constant weight at 80°C.

To estimate tissue distribution, chloroquine phosphate, 50 mg drug/kg body weight was administered orally as described previously to 18 fish. A control group of fish was maintained and treated similar to the other group, except that drug was not administered. Fish were necropsied and gill-, liver-, intestine-, plasma-, kidney- and muscle-tissues removed and frozen -70°C.

To estimate depletion dynamics of circulation drug levels, 9 fish received 50 mg drug/kg body weight. Mucous and 1 ml blood samples were obtained from each of two fish, 6 hr, 2-, 7-, 15-, 22- and 49-days after oral drug administration. Mucous and tissue preparations were processed for fluorimetric determination of chloroquine based upon the procedure described by Adelusi and Salako (1980). Fluorimetric reference standards consisted of 0, 5, 10, 20, 50 and 100 ng chloroquine phosphate ml in 0.1 M HCl. Standard tissue references were prepared using 2×

concentrations of the fluorimetric standards, mixed with equivalent (w/w or w/v) quantities of control tissues.

RESULTS AND DISCUSSION

When maintained in Ott's medium at ambient conditions (20°C), the majority of the test dinospores were active for as long as 96 hr. Exposure of the dinospores to chloroquine phosphate for 24 or more hours, at concentrations 2.5 μg/ml or greater resulted in almost complete loss of activity (Fig. 1).

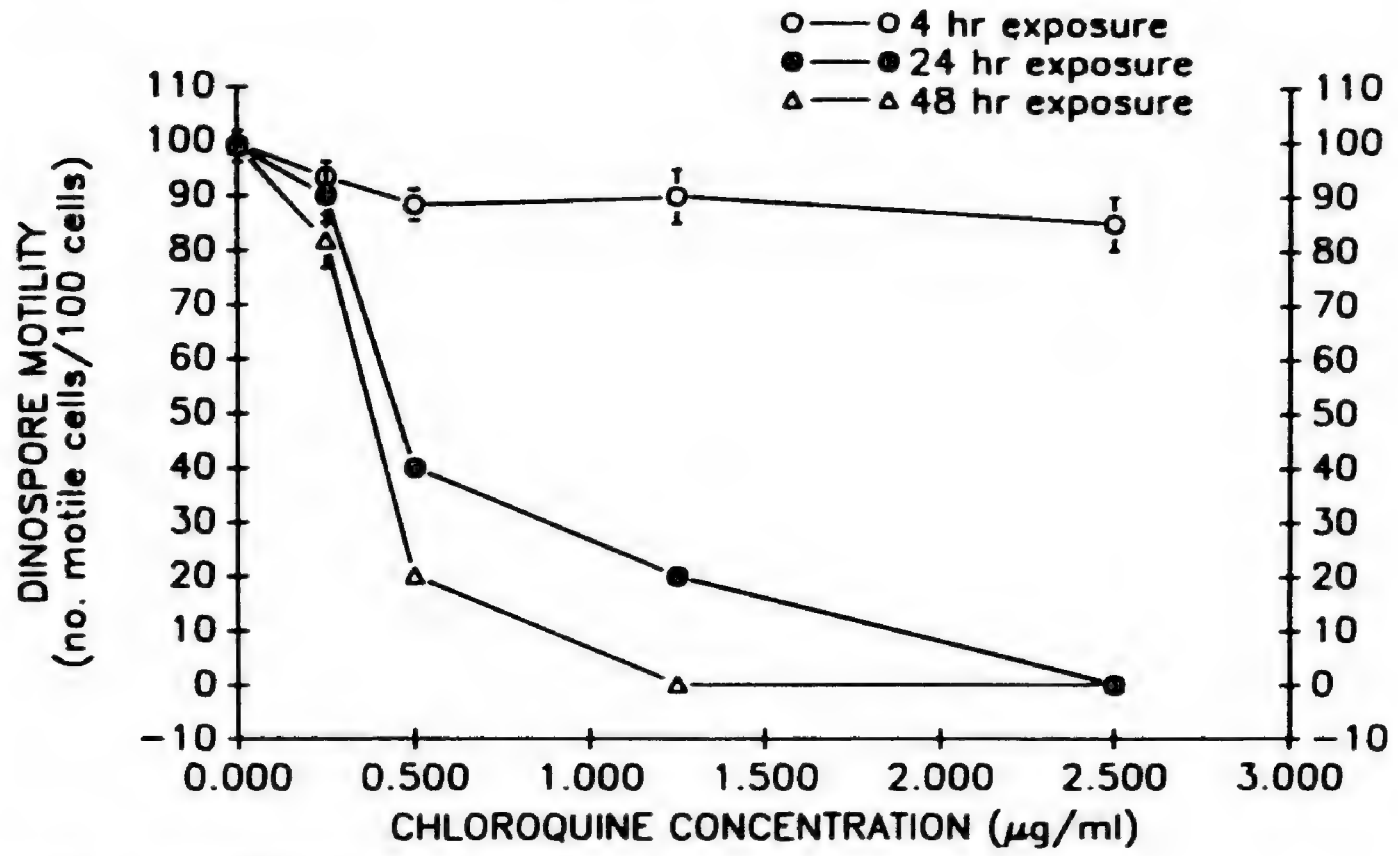

FIG. 1. Dinospore inhibition by chloroquine.

Comparable loss of activity following 4 hr exposure was observed in preparations exposed to 25, 125 and 250μg drug/ml, yet inhibition was not as pronounced at 500μg and 1000 μg/ml levels during the 4 hr exposure interval (Fig. 2). Levels of chloroquine which produced acute toxic affects to the experimental fish were not identified in this study. Adverse signs were not demonstrable in fish receiving at least 200 mg chloroquine/kg (approximately two times the lethal dose for man (Hong 1976)).

Linearity of the fluorimetric assay for chloroquine ($r = 1.00$; $y = 0.937 + 4.133\ x$) is demonstrated in Fig. 3.

The relationship between oral dosage and concentrations of the drug in gill mucous 24 hr after administration (Fig. 4) reveals that inhibitory drug levels (> 5 μg drug/ml) can be elicited by administering 10 to 70 mg drug/kg body weight. Administration of 50 mg drug/kg body weight results in distribution of inhibitory drug levels in all major fish tissue systems (Fig. 5). Data on

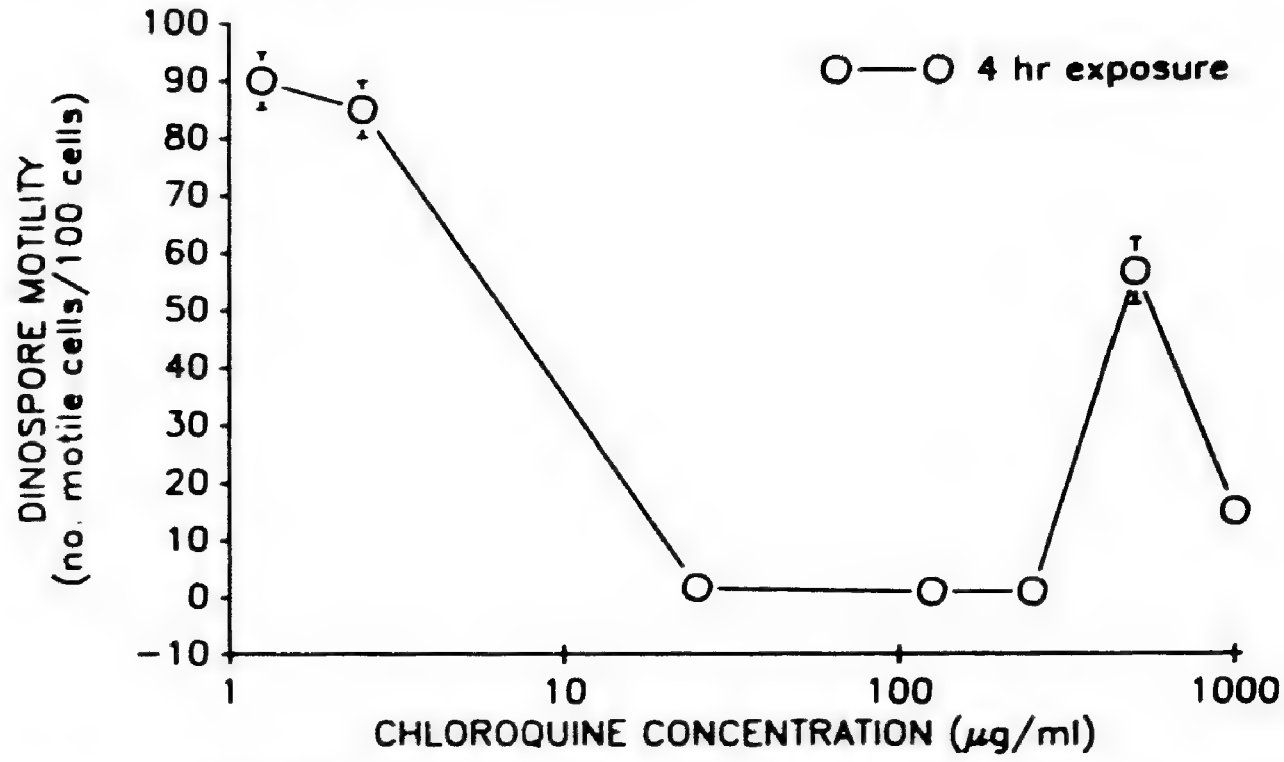

FIG. 2. Inhibitory effects of chloroquine (4 hr. exposure)

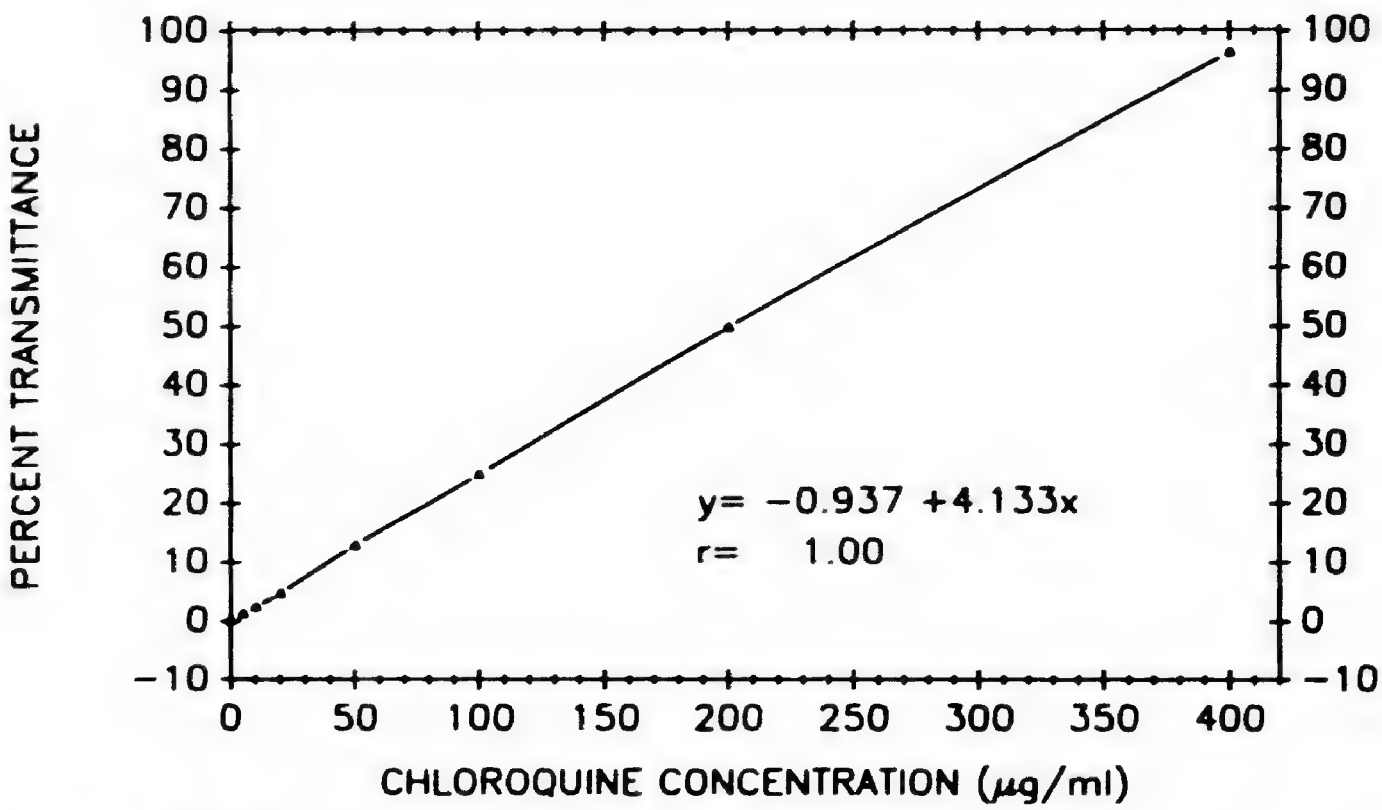

FIG. 3. Reference plot for fluorimetric assay.

circulating (plasma) and gill mucous drug levels following a single 50 mg/kg dose (Fig. 6) suggests that; a) inhibitory levels persist for at least 7 days in the fish and b) most of the drug disappears within 30 days.

Based upon the data of the present study, chloroquine would appear to be an effective drug for controlling amyloodinosis in red drum. The use of an oral therapeutic approach would result in tissue drug levels that would overcome

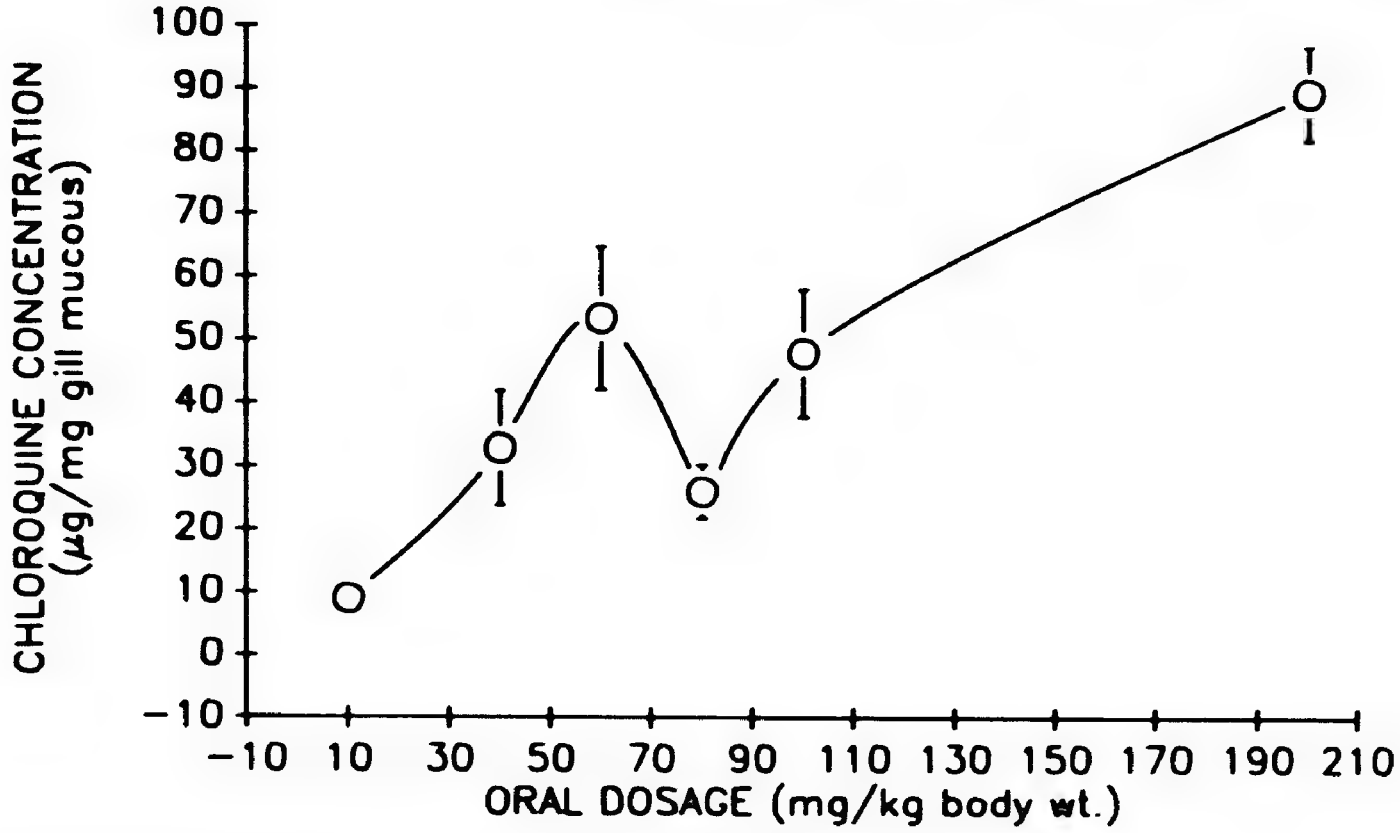

FIG. 4. Chloroquine levels in mucous 24 hr after administration.

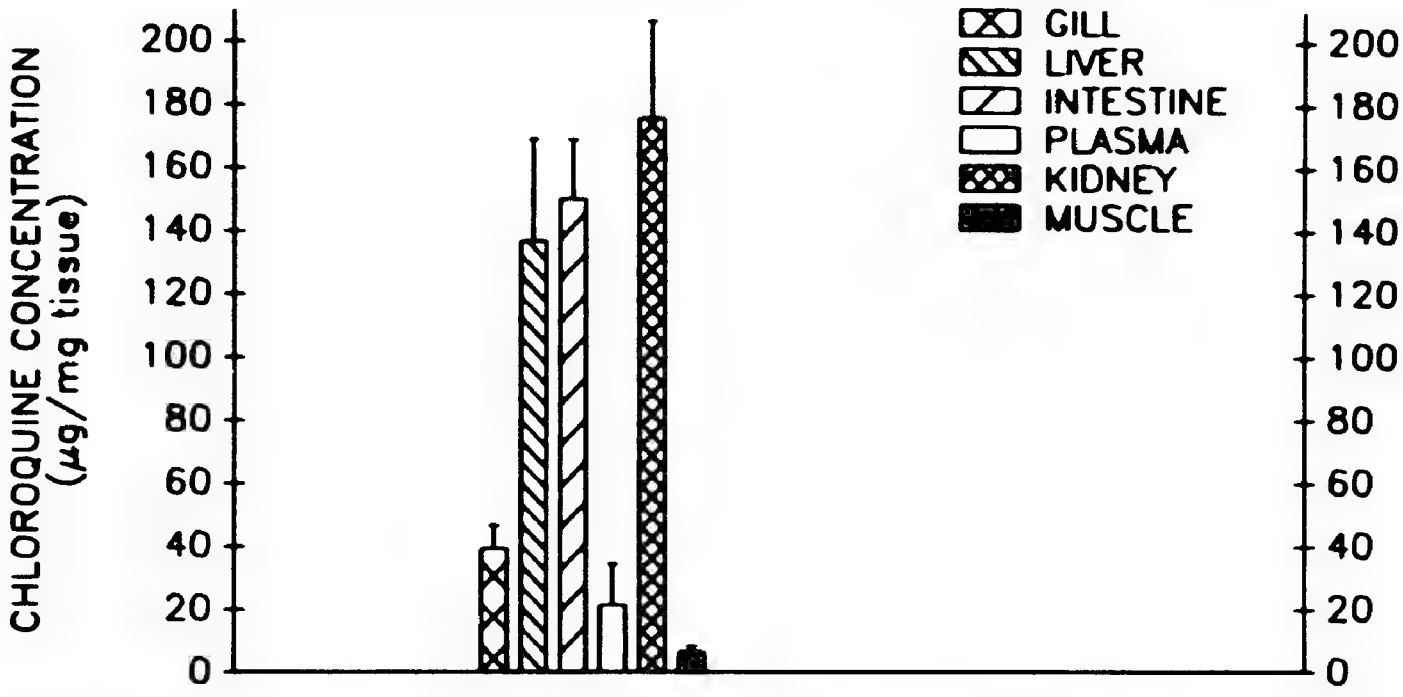

FIG. 5. Tissue distribution of chloroquine 24 hr after administering 50 mg kg.

some of the problems associated with current therapeutic practices (bath treatment) for this serious parasite (*e.g.* inhibition of microbial ecology of biofiltration system, effects on fecundity and reproductive performance of cultured fish, etc.). Inasmuch as the drug has been used as an antimalarial drug in human beings for more than four decades and has a relatively short tissue decay rate (< 45 days) in red drum, the drug would appear to offer promise as a safe therapeutic for amyloodinosis.

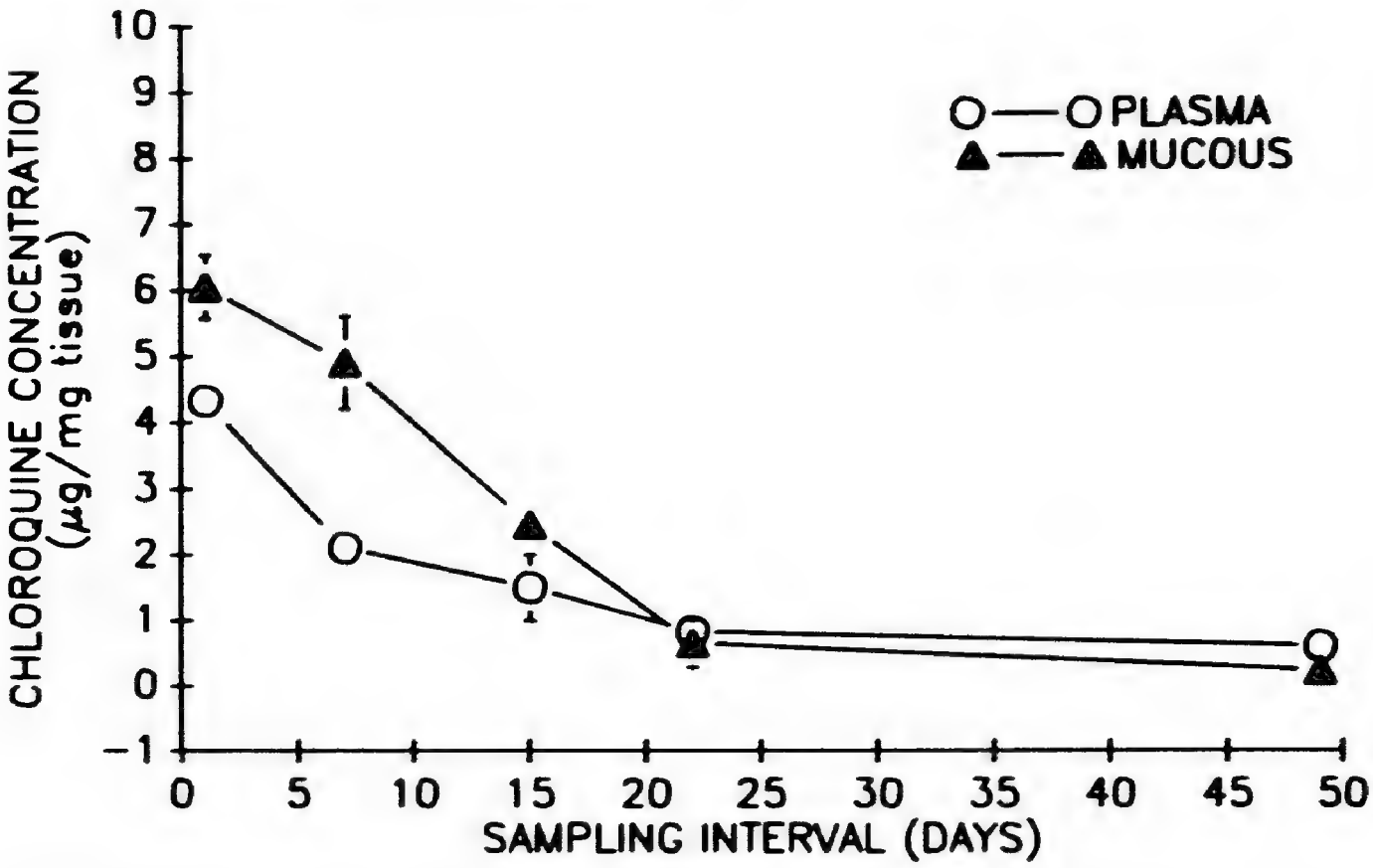

FIG. 6. Circulating levels of chloroquine after administering 50 mg kg body weight.

ACKNOWLEDGMENTS

The activities described in this study were supported in part by the Texas Agricultural Experiment Station project "Diseases of Aquatic Food Animals" (H-6011), the Texas Advanced Technology research Program "Commercial Development of Redfish Aquaculture". The generosity of the First Institute of Oceanography, State Oceanic Administration, Quingdao, China in providing technical consultation, and the technical assistance provided by Mrs. W.M. Charanza of the Department of Veterinary Microbiology and Parasitology, Texas A&M University are gratefully acknowledged.

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SECTION V

Abstracts of Papers not Included in Proceedings

DEVELOPMENT OF MARINE FINFISH CULTURE IN TEXAS: INTENSIVE CULTURE SYSTEMS

C.R. Arnold
The University of Texas at Austin, Marine Science Institute, Port Aransas, Texas 78373

The conventional method of growing fish and shrimp has been in outdoor ponds. The densities achieved by this method are meager compared to the new high density recirculating systems being developed by this research program. A new concept in water quality management and a new larval fish food is currently being tested on a pilot plant scale.

The results of this past year's research are very promising. The high density recirculating grow-out system as well as the new spawning facilities are performing better than anticipated. The water quality and the density of the grow-out system are such that commercial application is already in progress by private industry. Refinement of these systems is progressing at a rapid rate.

LABORATORY STUDIES OF RED DRUM AND LARVAE: A REVIEW

G. Joan Holt
The University of Texas at Austin, Marine Science Institute, Port Aransas, Texas 78373

Studies of marine fish development have suffered because of the difficulty in collecting these very sensitive organisms without damaging them in collecting nets. Since early stages of marine fish are small and can not easily be captured alive, their identification has been difficult and information on occurrence and distribution in natural systems lacking. During the last ten years a great many species have been identified but still many, particularly in the Gulf of Mexico and Caribbean, remain to be described. Fastest advances in this direction have occurred because of recent advances in spawning and rearing fish in the laboratory. Over the past few years we have conducted numerous studies of lab spawned fish to examine the effects of physical, chemical and biological factors on survival growth and development of larval red drum (*Sciaenops ocellatus*) and other sciaenids. In these studies we determined that temperature, salinity, ammonia, day length, prey density and fish density all affected growth.

Although variation due to these external factors can explain size differences among different populations, large size variations also occur within a population or tank of sibling fish. This phenomenon is due in part to genetic differences among individuals and in part due to social interactions and perhaps differential success at first feeding.

Development is closely tied to size, while age is less useful in predicting development stage. Fin development proceeds from caudal to dorsals, anals and pelvics. The first fins present in newly hatched larvae, the pectorals, are among the last to complete ray formation. Scales begin to form in 10 mm fish but scalation is not complete until 25 mm. Growth rate and fin development of lab reared red drum and wild-caught fish, aged by counting otolith "daily growth" rings, were found to be comparable. Eggs collected from plankton samples are similar in size, development rate and time of release to laboratory spawned red drum.

DEVELOPMENT OF MARINE FINFISH CULTURE: ARTIFICIAL DIETS FOR LARVAL FISH

G. Joan Holt
The University of Texas at Austin, Marine Science InstitutePort Aransas, Texas, 78373

A major factor limiting culture of marine fishes on a commercial scale is the lack of practical and economic diets for larval fish to replace culture of live foods now required. In the United States relatively little research effort has been applied to larval fish nutrition, perhaps due to an historic lack of commercial interest in marine fish cultivation. Counter to this, in the last few years a tidal wave of interest in mariculture has developed partially as a result of modern

demands for high quality, lowfat seafood diets and also due to research breakthroughs that have increased the prospect for commercialization of mariculture. Microencapsulation is currently experiencing widespread development and diversification for use in industries such as foods, pharmaceuticals, pesticides and carbonless paper. The larval fish food of the future may well be a micro-package of highly nutritious food encapsulated in a protective membrane.

During this past year a system to evaluate artificial diets for larval fish was designed and built. Installation of this system is now complete and feeding experiments have been initiated. Microcapsule design, currently underway, incorporates technology used in cell culture for large scale antibody production. Alginate gelation holds great promise for larval diets because the final product is very similar to their natural diet. Major factors that may be overcome by use of this ionic gelation process include capsule size, stability, palatability and buoyancy.

DYNAMICS OF ELEMENTAL AND BIOCHEMICAL COMPONENTS IN THE EARLY LIFE STAGES OF RED DRUM (*SCIAENOPS OCELLATUS*)

W.Y. Lee, S.A. Macko, X.H. Mao and C.R. Arnold
The University of Texas at Austin Marine Science Institute, Port Aransas, Texas 78373

This study describes how red drum manage to develop and grow during its early life stages. Dry weight, total carbon, nitrogen, hydrogen, protein, amino acids and lipid contents were determined for eggs, yolk-sac larvae and active-feeding larvae. During egg development, dry weight progressively decreased but size of the eggs remained unchanged. At hatching, the newly hatched larvae had lost 43% of their initial dry weight (40 mg), which corresponded to 42% carbon loss and 46% nitrogen loss. Both weight and energy content of the larvae continued to decline until day 6, at which time the fish had lost another 40% carbon and 14% nitrogen of the newly hatched larvae. After day 6, fish larvae grew rapidly and at a rate > 30 mg day (dry weight). In general, changes in total carbon content (40-51%) during the early life stages were much greater than in hydrogen (6.0-7.6%) and nitrogen (9.2-11.3%). Total percentage carbon is highest in eggs when development solely depends on the reserved energy of egg yolk, and it drops to minimum of 40% immediately after the yolksac is fully absorbed. Larvae older than 6 days feed mainly on extraneous particles, and contain about 41% of carbon, which remains fairly constant thereafter. Hence, C N ratios of red drum larvae vary accordingly with both life stages and energy reserves, with highest value (5.0) recorded for eggs and very stable values (*ca.* 3.7) for larvae older than 6 days. Other chemical components were monitored in a similar way as that of C, H, and N, and the significance of their changes at the early life stages of red drum will be discussed.

REGIME SPAWNING OF RED DRUM, *SCIAENOPS OCELLATUS*: CYCLICAL EFFECTS ON SERUM STEROIDS, GONADAL CYTOLOGY AND ULTRASTRUCTURE

Daniel E. Roberts, Jr., R.O. Reese, H.J. Grier, and R. MacGregor, III[1]
Florida Department of Natural Resources, Bureau of Marine Research,
St. Petersburg, Florida 33701
[1] Espey, Huston & Assoc., Inc., Galveston, Texas 77550

Gametocyte maturation in both male and female red drum, *Sciaenops ocellatus*, through spermiation and ovulation, respectively, was induced out-of-season using photoperiod and water temperature as exogenous stimuli. Oocyte cytology and ultrastructure, as well as serum androgen and estrogen, were evaluated biweekly and triweekly. Mean estrogen levels were highest (2000-4000 pg ml) in females after oocyte regression following spawning (week 6-9). Regression and atresia occurred during increasing photoperiod and temperature and after 14 weeks of sustained spawning at low photoperiod and temperature (10HL:20-23°C). Mean androgen levels in females were slightly elevated (200 pg/ml) two weeks prior to spawning (weeks 19-21). Mean androgen levels were more pronounced in females (200-800 pg/ml) and

males (200-700 pg/ml) during the spawning phase (weeks 22-35). Mean androgen levels in females fluctuated significantly during spawning. Elevated androgen levels in males were associated with spermiation. During this period proximal portions of testis tubules were filled with stored sperm and few cysts of developing spermatocytes were observed. When androgen levels were low (200 pg/ml) the testis was regressed. Elevated androgen levels in females (200-800 pg/ml) occurred when oocyte maturation, resumption of meiosis, and ovulation were ongoing in the ovary. Androgen levels decreased in males and females as atresia progressed. Changing ultrastructure of oocytes and spermatocytes and concomitant variations in endogenous factors (androgen and estrogen) were related to exogenous factors (photoperiod and temperature).

SEASONAL CYCLES OF OOCYTE GROWTH AND PLASMA STEROID HORMONE LEVELS IN CAPTIVE ORANGEMOUTH CORVINA BROODSTOCK

Peter Thomas and J.A. Prentice[2]
The University of Texas at Austin, Marine Science Institute, Port Aransas, Texas 78373
[2] Texas Parks and Wildlife Department, Heart of the Hills Research Station, Ingram, Texas 78025

Efforts to artificially breed orangemouth corvina (*Cynoscion xanthulus*), an important sciaenid sport fish in the Salton Sea, California, have been hampered by a lack of information about the reproductive biology and endocrinology of the species. The seasonal changes in oocyte growth and blood steroid hormone levels were monitored in captive female adult orangemouth corvina subjected to a condensed (8-month) seasonal cycle of photoperiod and water temperature. Intraovarian samples were collected by catheter and the diameters of the oocytes measured with an ocular micrometer to assess oocyte growth. Blood steroid hormone levels were determined by specific radioimmunoassays.

Plasma concentrations of the ovarian steroids, estradiol-17 and testosterone, began to increase in females when the water temperature and day length were maximal (28°C and 15 h, respectively). Circulating estradiol-17 and testosterone titers continued to rise as the temperature and day length decreased and reached maximum values (0.7 and 0.3 ng $ml.^{-1}$ respectively) during fall conditions. This elevation in the steroid levels was accompanied by rapid growth of the oocytes. Spawning occurred after two months of exposure to fall conditions at a time when plasma estradiol-17 and testosterone levels were still elevated. However, circulating levels of these gonadal steroids fell dramatically after spawning and reached minimum levels one month later. The significance of these results will be discussed in relation to the endocrine control of ovarian recrudescence in orangemouth corvina and how these findings may apply to sciaenid fishes in general.

A COMPARISON OF BLACK DRUM, RED DRUM, AND THEIR HYBRID IN SALTWATER POND CULTURE

Anne Henderson-Arzapalo, Robert L. Colura and Anthony F. Maciorowski
Texas Parks and Wildlife Department, Marine Fisheries Research Station, Palacios, Texas 77465

Hybridization has been used to advantage in aquaculture by providing fishes that exhibit improved growth and survival over the parent species. Previous studies demonstrated an intergeneric sciaenid hybrid could be successfully produced by crossing a female black drum (*Pogonias cromis*) with a male red drum (*Sciaenops ocellatus*). However, existing data were insufficient to determine if the hybrid offered any production advantages over red or black drum in extensive pond culture. In the present study, the two drum species and their hybrid were artificially spawned and simultaneously pond cultured over a nine month period. Two to three day old fry of each type were initially placed into three separate 0.1 ha nursery ponds. After 43 days, nursery ponds were harvested and 6,000 fingerlings of each type retained for transfer to six

replicate 0.2 ha ponds. Two ponds received black drum, two ponds received red drum, and two ponds received hybrid drum. Each pond was stocked with 3,000 fingerlings, fed a commercial feed, and harvested after 235 days. At harvest, individual black drum averaged 144.4 g and 214 mm (TL). Respective values for red drum were 143.2 g and 236 mm, whereas hybrid drum averaged 190.1 g and 245 mm. Percent survival over the production period was 94, 63, and 72%, respectively, for black drum, red drum, and hybrid drum. Production for the respective species was 10.6, 7.0, and 10.7 kg ha day. A taste panel comparison indicated no significant flavor differences between the three fishes. Hybrid drum exhibited faster growth and more efficient food conversion than red drum. However, black drum provided the best overall survival. These data suggest hybrid drum, red drum, and black drum all have mariculture potential.

DISTRIBUTION OF EGGS AND LARVAE OF RED DRUM (*SCIAENOPS OCELLATUS*) IN TEXAS COASTAL WATERS

S.A. Holt, G. Joan Holt and C.R. Arnold
The University of Texas at Austin, Marine Science Institute, Port Aransas, Texas 78373

Aspects of the early life-history of red drum (*Sciaenops ocellatus*) have been the subject of several research projects and long-term investigations. Red drum eggs have been taken from early September through mid-October in the Gulf of Mexico within one mile of the Aransas Pass in Texas. Egg densities as high as 2000/100 m^3 have been observed but densities of 200-300 eggs/ 100 m^3 are more typical. A few red drum eggs have been taken in a limited number of samples taken farther offshore. Likewise, no eggs have been taken inside the estuary except in Gulfwater which moves into the lower reaches of Aransas Bay on flood tide. There is no evidence of spawning in the estuary. The distribution of planktonic red drum larvae is similar to that of eggs. The highest densities are found offshore in 30-50 m water depth within 1 mile of the tidal pass. Densities of 30-40 larvae 100 m^3 are typical from mid-September through October. Red drum larvae are present in similar densities in the Aransas Pass in "Gulf water" but are rarely captured in bay water. Densities are similar on surface and bottom in the tidal channel on flood tide but are greater on the bottom during ebb tide, suggesting use of "selective tidal stream transport" as a mechanism for movement of larvae and postlarvae from offshore spawning area into the estuarine nursery grounds.

DEVELOPMENT OF MARINE FINFISH CULTURE IN TEXAS: IMMUNIZATION AGAINST DISEASE

D.H. Lewis
Texas A&M University, Veterinary Microbiology Department, College Station, Texas

Effective treatments to prevent outbreaks of infectious diseases in marine fishes need to be developed before large scale production is attempted. The long term aim of this research program is to develop effective vaccines or treatments for all the major infectious diseases encountered by cultured marine fishes in this region.

Considerable progress has been made in our understanding how red drum respond to infectious diseases and in the development of immunization methods for the disease prevention. Red drum showed a strong immune response to three types of antigens: viral (bacteriophage), particulate (bovine red blood cells) and soluble (bovine serum albumin). These results suggest that vaccination protocols, similar to those used with other domestic species, would be applicable to controlling disease problems in red drum. Currently vaccines to control *vibrio* and *oodinium* infestations in red drum are being developed and tested.

BIOCHEMICAL STRESS RESPONSES OF RED DRUM TO COMMON CULTURE PROCEDURES

Peter Thomas
The University of Texas at Austin Marine Science Institute Port Aransas, Texas 78373

Cultured fish are reared under conditions which are rarely optimal, particularly in intensive culture systems where water quality may be poor, nutrition inadequate and the fish crowded. In addition, common aquaculture procedures such as handling, confinement, transport and disease treatments usually cause a variable degree of trauma to fish. These adverse environmental stressors are often associated with reduced growth and increased susceptibility to disease.

The purpose of the present study was to determine the effect of certain common fish culture procedures on the plasma corticosteroid and glucose stress responses of red drum. Capture and handling elicited rapid, marked elevations of plasma cortisol and glucose in fish. The responses were transient, however, and the plasma concentrations returned to initial values one day after handling. Similarly, transportation of red drum induced rapid but transient plasma cortisol, glucose and osmolality stress responses. Chronic elevation of these parameters was noted during an outbreak of disease. The effects of disease treatments on these biochemical stress responses in red drum was also investigated. Methods to assess the effects of stressors and to reduce biochemical stress responses in cultured fish will be discussed.
Preliminary Information. Not For Citation.

EVALUATION OF METOMIDATE AS AN ANESTHETIC FOR COMMON SNOOK, *CENTROPOMUS UNDECIMALIS*

G.K. Vermeer and B. Falls
Florida Department of Natural Resources, Bureau of Marine Research, 100 Eighth Avenue, St. Petersburg, Florida 33701

Metomidate (an analog of etomidate) is a potent, hypnotic anesthetic. Induction and recovery times of juvenile and adult snook exposed to metomidate were recorded. Adult mean standard lengths were 63.3 cm and juveniles were 16.6 cm. Induction time was defined as the time from first contact with the anesthetic until equilibrium was lost and swimming motions ceased. Recovery time was the time interval to return of equilibrium after immersion in drug-free seawater. Long term recovery was evidenced by a return to normal feeding behavior. Adult snook induction times at 8 mg/L metomidate were 162′ 16″ (x′ S.E., n = 21) and recovery times were 346′ 49″. Juveniles at the same anesthetic concentration had induction times of 72′ 6″ (x′ 1 S.E., n = 17) and recovery times of 121′ 19″. Adult induction and recovery times were more than twice as long as juveniles, however all times were well within accepted limits. Normal feeding behavior, after anesthesia, resumed within two days for juveniles and within four days for adults. Metomidate is a safe, effective and low-stress anesthetic for snook.